Poster Presentations
Antimicrobial resistance, phylogenetic distribution and molecular docking of integrons in multidrug resistant diarrheagenic E.coli isolates from children under five in Delhi, India.

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**Background:** Integrons are versatile gene acquisition systems commonly found in bacterial genomes. They are ancient elements that are a hot spot for genomic complexity, generating phenotypic diversity and shaping adaptive responses. Mobile gene cassettes captured within integron arrays encompass a vast and diverse pool of genetic novelty. These elements are able to capture and express gene cassettes encoding antibiotic resistance. The main aim of this study was to investigate the distribution of integrons in multidrug resistant diarrheagenic E.coli isolates, to analyze the possible relationship between the antimicrobial resistances profiles, phylogenetic grouping with presence of integrons and to perform molecular docking of integron proteins.

**Methods & Materials:** 80 diarrheagenic E.coli strains were isolated from children with diarrhea and processed by known conventional methods. All isolates were tested for antimicrobial susceptibility using CLSI guidelines followed by identification of their phylogroups by Clermont et al, 2013 method after extraction of genomic DNA. Presence of class 1, 2 and 3 integrons was seen by Real Time PCR. Docking was performed using various softwares like Marvin sketch, viewerlite, pymol, pardock. Statistical analysis was used for the comparison of the categorical data

**Results:** Class 1 integron was identified in 58.75% isolates while 18.75% isolates harbored class 2 integron and no class 3 integrons were detected in any of the isolate. 11.25% isolates showed co existence of class 1 and class 2 integrons. Integrons were significantly associated with resistance to certain antibiotics, including; Cefotaxime (P = 0.01), Ceftazidime (P=0.006), Azetronam (P=0.046), Nalidixic acid (P=0.01), Gentamycin (P=0.001), Amikacin (P=0.01) and Piperacillin+tazobactam (P=0.037). Docking of integron protein was performed with Cefotaxime and Ciprofloxacin.

**Conclusion:** Our study demonstrates the importance of integrons for the occurrence and transmission of multidrug resistance. Identical predominant class 1 and 2 integrons in E.coli servers' indicate horizontal transfer. This study emphasizes the alarming role of integrons in antibiotic resistance within diarrheagenic E. coli strains. Modeling and docking studies may provide useful insights for developing new antibiotic drugs to minimize multidrug resistance.
Correlation of $\beta$-lactam resistance with over expression of efflux pumps among neonatal septicemic isolates of Acinetobacter baumannii from India  
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**Background:** The emergence of multidrug-resistant *Acinetobacter baumannii* has created severe challenges for neonates hospitalized in NICU. *A. baumannii* strains have the propensity for developing antimicrobial resistances extremely rapidly. Neonatal sepsis is a common diseases caused by *A. baumannii* in a developing country like India. Emergence of $\beta$-lactam resistance makes the scenario more critical for neonates for whom treatment options are limited. Now-a-days efflux pumps are getting importance because of their wide substrate profile. Although their presence were scarcely reported but often work in concert to produce high antibiotic resistance. Our aim was to assess the role of efflux pumps in $\beta$-lactam resistance among neonatal septicemic *A. baumannii* isolates.

**Methods & Materials:** MIC values of carbapenems (meropenem, imipenem) and cephalosporins (cefotaxime, ceftazidime) for neonatal septicemic *A. baumannii* isolates were determined using broth dilution method with and without efflux pump inhibitor PA$\beta$N. Efflux pump genes of RND families (AdeABC, AdeIJK, AbeXYZ), and SMR family (AbeS) were confirmed by PCR followed by sequencing. Over expression of the genes was carried out by real time PCR. The regulatory components (*adeR* and *adeS*) of AdeABC pump was investigated for the isolates, over expressing *adeB* gene.

**Results:** Carbapenems and cephalosporins resistance was found among 61% and 90% of total *A. baumannii* isolates (n=49). Exposure of the isolates to PA$\beta$N resulted in $\geq 4$-fold MIC decreases for carbapenem and cephalosporins among 25% and 35% of the isolates which indicated the existence of multidrug efflux pumps. Overall, 55% of these isolates had shown over expression of *adeB* gene followed by *adeY, adeS*, and *adeJ* (Figure 1). The relative expression level for *adeB* was highest in comparison to the other pump genes. The presence and over expression of AdeXYZ efflux pump had never previously been reported among *A. baumannii* isolates. Specific mutations had been detected within *adeR* (V136A) and *adeS* (K121E) which might have positive effect on over expression of the pump.

![Figure 1. The relative expression level for RND families (AdeABC, AdeIJK, AbeXYZ) and SMR family (AbeS) efflux pump genes among neonatal septicemic *A. baumannii* isolates (Isolate 1 to Isolate 18).](image)

**Conclusion:** This is probably the first study that showed the active efflux pumps appeared to play important roles in the $\beta$-lactam resistance of *A. baumannii* isolated from neonates. Therefore, efflux pump inhibitors may be useful as adjunct therapy for treatment of these multidrug-resistant strains.
Assessment of anti-bacterial activity of silver ions in infected diabetic foot ulcers – An answer to antibiotic resistance

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Background: Easy accessibility and irrational use of antibiotics has led to emergence and spread of multidrug bacteria in nosocomial and community settings. MDR strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and Enterobacteriaceae are commonly isolated from infected diabetic ulcers. Escalating antibiotic resistance has led us to reconsider use of heavy metals particularly silver which is known to reduce bacterial burden in infected wounds. This study aims to assess the bactericidal and clinical effect of silver alginate foam dressings on infected diabetic foot ulcers.

Methods & Materials: Fifty cases of infected diabetic foot ulcers were studied. Quantitative bacterial cultures on sheep blood agar were performed as baseline (prior to treatment) and at 7 and 14 days following treatment with silver alginate dressing. Results of quantitative cultures were co-related with clinical improvement in ulcer size and local signs of infection. Bacteria isolated were identified and antibiogram was determined as per CLSI guidelines. Killing curves were recorded by inoculating the surface of silver alginate dressing with $10^5$ bacteria. Sensitivity and duration of silver ion activity was assessed by disc diffusion method on nutrient agar using 7mm disc of silver dressing. Growth inhibition zone diameters were recorded by serial transfer of silver ion foam disc onto freshly inoculated plates for 7 consecutive days.

Results: Bacteria isolated included *S.aureus* 32% (28% MRSA), *E.coli* 24% (39% ESBL producers, 12% MBL producers), *P.aeruginosa* 37% (15% MBL producers), *Enterobacter spp* 4% and *Citrobacter* 3%. Killing curves showed sterility approximately one to 2.5 hours following exposure to silver ions. All the isolates were sensitive to silver ions irrespective of their antibiotic resistance status. Growth inhibition zone diameters were maintained steady upto 7 days. In all cases decrease in bacterial load was associated with decrease in ulcer size.

Conclusion: This study shows topical use of silver ions could serve as an effective alternative to topical or oral antibiotics in management of infected wounds. When used once weekly silver alginate foam dressing appears to be effective in reducing wound bio-burden which is positively associated with ulcer healing. Thus we can conclude that silver ions can be used effectively for treatment of chronic ulcers.
Antimicrobial susceptibility pattern and sequence analysis of DNA gyrase and DNA topoisomerase IV in salmonella enterica serovars typhi and paratyphi A at a tertiary care centre in North India

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**Background:** The aim of this study was to determine the antimicrobial susceptibility pattern of typhoidal Salmonella isolates recovered from human infections as well as to investigate the association of quinolone resistance with mutations in the genes coding for DNA gyrase and topoisomerase IV.

**Methods & Materials:** The study was conducted at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India from January 2013 to September 2015. A total of 96 Salmonella enterica serotypes Typhi, Paratyphi A and B recovered from blood cultures in cases of enteric fever were included in the study. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion as well as E-test to the following agents: ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, nalidixic acid, ofloxacin, ciprofloxacin, levofloxacin and ceftriaxone. Results were interpreted as per the Clinical and Laboratory Standards Institute guidelines, 2015. Genotypic characterization included the screening of mutations in the quinolone resistance-determining regions (QRDR) of gyrA, gyrB, parC, and parE by PCR. Purified PCR reactions were sequenced by Sanger sequencing and analyzed by Clustal W multiple sequence alignment tool in 60 isolates.

**Results:** A total of 96 isolates of S. enterica serovars Typhi, Paratyphi A and B respectively were recovered during the study period. S. Typhi was the predominant serotype (n=77), followed by S. Paratyphi A (n=17) and S. Paratyphi B (n=2). Only 4 out of 77 S. Typhi were resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole while none of the Paratyphi A or B were multi-drug resistant. All S. Typhi isolates except two were nalidixic acid resistant (NAR). All Paratyphi A were NAR. Decreased ciprofloxacin susceptibility was seen in 63 out of 77 S. Typhi recovered (81.8%). Complete resistance to ciprofloxacin was observed in 12 isolates. No resistance to ceftriaxone was documented. The most common mutation in gyrA was at codon Ser83 to phenylalanine (n=34) or tyrosine (n=12). Five S. Typhi isolates that were resistant to ciprofloxacin had a second mutation at Asp87 to Asn in the gyrA gene.

**Conclusion:** The change in the susceptibility pattern of chloramphenicol, ampicillin, and cotrimoxazole is noteworthy. There is a need to review the use of fluoroquinolones for the management of enteric fever in endemic areas.
Is Moxifloxacin a secret weapon or simply a trump card to treat methicillin resistant staphylococcal infections? A study from Egypt

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Background: Staphylococcus is a leading cause of nosocomial and community acquired infections. The discovery of penicillins made the treatment of such infections possible. The widespread and improper use of these agents made them practically useless as bacteria developed resistance to their action, a fact that created the need for newer agents. Moxifloxacin (MOX), a fourth generation fluoroquinolone, is one such agent currently recommended for treating staphylococcal infections, including methicillin-resistant ones. In this study, we explored the effectiveness of MOX, alone and in combination with benzalkonium chloride (BZ), in treating methicillin-resistant staphylococcal ocular isolates obtained from pediatric outpatients in two hospitals in Alexandria, Egypt. We also studied the rate and magnitude of resistance development as a result of MOX use.

Methods & Materials: We determined the minimum inhibitory concentrations (MICs) of MOX and other antibiotics against the tested isolates, using the agar dilution technique. For laboratory induction of MOX resistance, we serially passed the susceptible isolates in medium containing increasing concentrations of MOX. We used time kill assays to assess the combination of sub-inhibitory concentrations of MOX and BZ against selected isolates.

Results: A total of 107 methicillin resistant staphylococcal ocular isolates were collected from patients suffering from conjunctivitis (n=75) or blepharitis (n=32). Moxifloxacin resistance was detected in 48.6% of the isolates. Of the MOX-susceptible isolates, 71.43% were resistant to ofloxacin, ciprofloxacin and levofloxacin. Upon laboratory induction of MOX resistance in a subset of the MOX-susceptible isolates, seven of the 15 (46.67%) MOX-susceptible isolates developed MOX resistance with 2 to 16-fold increases in MIC. The combination of MOX and BZ (a commonly used preservative in eye drops, but surprisingly not used in MOX preparations) resulted in a bactericidal synergistic combination with large reductions in survivors relative to control (>6 logs) and MOX (~2 logs) alone.

Conclusion: Compared to older fluoroquinolone members, MOX showed greater therapeutic potential and fewer resistance levels. However, the continued use of MOX is expected to trigger higher resistance rates. Therefore, and on the basis of their synergy, we recommend the use of BZ as a preservative in MOX eye drop preparations to retard resistance development.
Emergence, spread and exchange of blaNDM-1 gene among Enterobacteriaceae in septicaemic neonates

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Background: Treatment of neonatal sepsis has become a challenge with the emergence of carbapenemase-producing bacteria. In recent years, the proliferation of New Delhi Metallo-β-lactamases has changed the scenario significantly. NDM is a zinc–requiring MBL that can hydrolyse all penicillins, cephalosporins, carbapenems and spares only the monobactam aztreonam. NDM-1 is often associated with other antibiotic resistance genes and plasmids carrying blaNDM-1 can easily transfer this resistance to other bacteria. Current worldwide dissemination of the blaNDM-1 was not related to the spread of one or more specific clones, to the spread of specific plasmids, or to the dissemination of one given genetic cassette.

Methods & Materials: The trend of carbapenem resistance in our unit was studied over five year period (2007-2011). All Enterobacteriaceae isolated were identified and antibiotic susceptibility profile was evaluated along with phenotypic tests. Molecular typing and conjugation/transformation was performed only for carbapenemase-producing isolates. Further molecular characterization and genetic features including plasmid-typing, presence of insertion-sequences and analysis of integron-structures were carried out for carbapenem-resistant Enterobacteriaceae.

Results: Fifteen NDM-1-harbouring Enterobacteriaceae isolates were identified from blood of septicaemic neonates; no other carbapenemases were identified. NDM-1 producing isolates were resistant to other broad-spectrum antibiotics and possessed ESBLs, AmpCs, 16S-rRNA methylases, AAC(6’)-Ib-cr and class 1 integron. Pulsed field gel electrophoresis indicated that the isolates were clonally diverse (Figure 1); hence, conjugal transfer of blaNDM-1 was investigated. NDM-1 was harboured by different plasmid scaffolds (FIA/FIB, L/M, FII, R, N, FIIK, HIB-M/FIB-M and nontypable) which were successfully transferred to E. coli J53 strain. Genetic structures surrounding the blaNDM-1 showed that at least a remnant of insertion sequence ISAba125 was methodically present upstream of the blaNDM-1. The entire ISAba125 element was identified upstream of the blaNDM-1 gene in most isolates. Downstream of the blaNDM-1, the bleMBL gene was quite systematically identified. The variable region of IntI1 presented different combinations of aacA4, dfrA5, dfrA12, aadA2, arr-3, dfrA1 and blaCARB-2, but no NDM-1.

Conclusion: This work underlines how efficient the spread of the carbapenemase gene could be among Enterobacteriaceae. It also throws light on the association of multiple resistances and genetic diversity of the NDM-1 in a neonatal unit of a developing country.
Comparison of antimicrobial resistance determinants and Staphylococcal Cassette Chromosome mec elements of Staphylococci isolated from human and veterinary origin

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**Background:** Staphylococcal species causing human infections have developed resistance to almost all the antimicrobial agents in clinical use today. In recent years, there has been an increase in reports of the isolation of multidrug-resistant (MDR) *Staphylococcus* spp. in veterinary medicine. The development of MDR is primarily due to the acquisition of multiple antibiotic resistance genes and its association with mobile genetic elements such as SCCmec. Asymptomatic colonization with MDR staphylococci in animal handlers represents a major risk factor for transfer of antibiotic-resistant determinants from animals to humans or vice versa. Hence, this study is aimed to compare the distribution of resistance determinants and SCCmec amongst clinical staphylococcal isolates from human and veterinary origin and carrier isolates of animal handlers.

**Methods & Materials:** A total of 144 staphylococcal isolates were included for the study, viz., 48 clinical isolates of veterinary origin [Group-I], 47 human carrier isolates from animal handlers [Group-II] and 49 clinical isolates of human origin [Group-III]. Antibiotic sensitivity testing was carried out for routinely used antibiotics. Detection of determinants implicating resistance to β-lactams [blaZ, mecA], macrolides [ermA, ermC, msrA], tetracyclines [tetK, tetM], aminoglycosides [aac(6’)-le-aph(2’), ant(4’)-la, aph(3’)-IIla] and trimethoprim (dfrA) were amplified using PCR. Further, SCCmec typing was done to determine the genetic diversity of the isolates.

**Results:** Out of 144 isolates, 78 isolates (54%) were methicillin resistant. Resistance to penicillin was highest (72%) followed by quinopiristin (43%), trimethoprim-sulfamethaxazole (41%), tetracycline (38%), erythromycin (33%), ciprofloxacin (30%), clindamycin (24%) and gentamicin (16%). Percentage of MDR strains was highest in Group-III (61%) followed by Group-II (53%) and Group-I (46%). **blaZ**, **mecA**, **ermC**, **msrA** & **tetK** showed highest prevalence in human carrier isolates, while **dfr**, **aac(6’)-le-aph(2’)** & **aph(3’)-IIla** were predominant in human clinical isolates and **ermA** in animal clinical isolates. Isolates of Group I & Group II predominantly exhibited **SCCmec** type I while, **SCCmec** type V in Group I. Combinations of **SCCmec** types were exhibited by isolates of Group II.

**Conclusion:** Amongst the study groups, carrier isolates from animal handlers are found to carry highest resistance determinants and combinations of **SCCmec** types indicating their acquiring these from both human and veterinary source and are potentially at risk of infections.
The impact of pre-hospital antibiotics on blood culture yields in a low resource setting

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Background: Bacterial sepsis and severe focal infections are common admission diagnoses amongst Gambian children admitted to the Edward Francis Small Teaching Hospital (EFSTH) and the Medical Research Council (MRC) Unit, The Gambia. However, blood cultures are frequently negative. To understand the causes of the low blood culture yields we documented history of antibiotic use and measured antimicrobial activity in clean catch urine collected at the time of admission or before the start of antibiotic therapy.

Methods & Materials: Children aged between 1 month to 18 years presenting with sepsis or severe focal infections were recruited into the European Union Childhood Life-threatening Infectious Diseases Study (EUCLIDS) at the EFSTH and MRC wards. Participants were interviewed for history of antibiotic use in the last seven days. A sterile Whatman No.1 filter paper disc (6mm) was inoculated with 20ul of patient urine collected pre-antibiotics and placed on a Mueller–Hinton agar plate pre-streaked with antibiotic sensitive Staphylococcus aureus (ATCC 25923 strain). After 18-24hrs incubation at 35-370C, growth inhibition around the disc was considered evidence of pre-hospital antibiotic exposure.

Results: Of 253 cases recruited, 128 (40%) were from MRC and the rest from EFSTH. Half of the cases were less than five yrs old. 182 (72%) were diagnosed with sepsis or focal infections with no organism identified. An organism was identified in 71 (28%) cases with the most common isolates Staphylococcus aureus 26 (35%), Streptococcus pneumoniae 13 (18%) and Neisseria meningitidis 10 (14%). Patients with osteomyelitis and meningitis were most likely to have a positive culture (80% and 46% respectively). The antimicrobial results did not tally with patient reports of antibiotic use: only 91 (36%) reported antibiotic use while antimicrobial activity was detected in 197 (78%).

Conclusion: A high rate of pre-hospital antibiotics in this setting makes conventional microbiology a poor diagnostic tool for sepsis and focal infections. The urine antibiogram assay is a sensitive and cheap approach for detection of antimicrobial activities and may serve as a useful tool for assessing the accuracy of antibiotic reports. There is an urgent need for more sensitive and cost effective molecular diagnostics for bacterial detection in low resource settings.
Vaccination of stray dogs against rabies is an effective strategy to reduce the risk of human rabies

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Background: Human Rabies is mainly infected through the dog bites in Sri Lanka. Mass vaccination against rabies and surgical sterilization are new strategies implemented for control of rabies in stray dogs after 2006 instead of mass culling. WHO recommends 70% vaccination coverage for eradication of rabies in dog population. Objectives were to survey stray dog population in Municipality, Dehiwala area (21 km²) and to assess the effect of two consecutive mass vaccination of them against rabies.

Methods & Materials: 8 wards out of 29 wards were selected randomly as samples for surveillance. Counting was carried out five days between 6.30 am. and 9 am. in 8 wards. Road maps of the respective wards were utilized to identify the boundaries and to cover the entire roads of the respective ward. Dogs visible on the road at the time of counting were only considered. After completion of surveillance, vaccination was carried out ward by ward in a sequence to cover the entire 29 wards from October 2013 to March 2014 and from October 2014 to March 2015 for 1st and 2nd round vaccination respectively. Red and blue collars were used for identification of vaccinated dogs for 1st and 2nd round respectively. Dogs were caught with the aid of catching nets. After subcutaneous administration of vaccine (Rabisin®) dogs were released to same locality.

Results: Estimated stray dog population is 1398 (CI±386). First and second round vaccination coverages were 97% (1364) and 88% (1231) respectively. 17 rabid dogs were reported in 2013. After vaccination commenced, the prevalence of rabid dogs in 1st, 2nd and 3rd quarters of 2014 was zero. 2 positive cases were reported only for 4th quarter of 2014. Positive rabid cases were not reported up to 3rd quarter of 2015.

Conclusion: Pre-exposure vaccination with the phenomenon of 'herd immunity' has marked impact on prevalence of canine rabid cases. Making comprehensive multi year vaccination plans with reliable, high quality vaccine of prolong immunity can improve herd immunity of dog population to reduce the risk of human rabies.
Follow up with systematic revaccination and vaccination of additions to dog population are also important to maintain herd immunity.
Mosquito infection with dengue and yellow fever in Bayelsa and Benue States, Nigeria

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Background: Dengue (DEN) and yellow fever (YF) are caused by two most important arboviruses in Nigeria. Outbreaks have been previously reported for YF virus without identification of the vector(s) of the disease. The use of molecular tool is scarcely applied as this will not only provide a near accurate result as to the detection of infection in mosquito, but will ensure that reliable field information on infection rates is accurately captured. This is central in precisely targeting the vector(s) responsible for YF or DEN transmission so as to avoid an upsurge and possibly an outbreak. In Benue, there is a history of YF-outbreak; and our result on entomological risk assessment foresees resurgence.

Methods & Materials: A total of 164,085 female adult mosquitoes were caught and pool-screened using polymerase chain reaction for YF and DEN infections in two Nigerian States (Benue and Bayelsa) between 2010 and 2011. Similarly, a total of 431,381 larvae were collected in only Benue for entomological risk assessments (house, container, Breteau indices) with the determination of the transovarial status of some immature Aedes mosquitoes across studied locations.

Results: In Benue, Aedes luteocephalus, Ae. aegypti and Anopheles gambiae were positive for YF. Meanwhile no mosquito was positive for DEN virus in Benue. For Bayelsa, only Mansonia africana was positive for DEN-3 virus as against negative results of all screened mosquitoes for YF. Entomological risk indicators suggest that three (Oju, Ega and Otukpo) of the four communities surveyed in Benue are at the verge of YF-epidemic. Evidence of a possible transovarial transmission was seen in Ae. aegypti from Ega only.

Conclusion: These communities should be placed on a high alert of a possible epidemic; and so, urgent step to clear the areas of potential mosquito sites is highly recommended.
Background: The dermatrophic variant of L. donovani causes cutaneous leishmaniasis (CL) in Sri Lankan patients. Standard treatment is painful, costly, repeated intra-lesional (IL) injections of sodium stibogluconate (SSG). Treatment failures are increasingly reported, hence the need to investigate alternatives. Thermotherapy is a tested treatment for L. tropica and L. major CL. Efficacy, safety and cost-benefit of thermotherapy were assessed for the first time, for L. donovani CL.

Methods & Materials: Laboratory-confirmed CL patients with single lesions were randomly assigned to (i) test group (n=98; received a single session of radio-frequency induced heat therapy (RFHT) at 50ºC for 30 seconds) and (ii) control group (n=115; received weekly IL- SSG until cure or 10 doses). Patients were followed-up fortnightly for 12 weeks to assess clinical response and adverse events. Cost of treatment was assessed using the scenario building technique.

Results: Cure rates by 8, 10 and 12 weeks in the thermotherapy group were 46.5%, 56.5% and 65.9% as opposed to 28%, 40.8% and 59.4% in IL-SSG group. Cure rate by thermotherapy was significantly higher (p=0.009) at 8 weeks and (p=0.035) at 10 weeks, while comparable thereafter. Response to thermotherapy at 8 weeks was significantly higher in females [OR 1.93 (95% CI 0.997-3.738)], papular lesions [OR 2.73(95%CI 1.29-5.77)] and in lesions <2cm [OR 1.95 (95% CI 0.98-3.87)] compared with IL SSG (p=0.05, p=0.009 and p=0.05). No major adverse events were recorded. It was 8.8 times cheaper to use thermotherapy (LKR164.00/patient) than IL SSG (LKR1453.00/patient).

Conclusion: A single application of thermotherapy was safe, cost-effective and convenient as compared to multiple doses of IL SSG in treatment of L. donovani CL.
Identification and functional validation of a biomarker for the diagnosis of miltefosine relapse during visceral leishmaniasis

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Background: Miltefosine is the only orally administrable drug for the treatment of leishmaniasis. It is used as a first line drug for the Kala-azar Elimination Programme in the Indian subcontinent. But in recent years, a decline in its efficacy points toward the emergence of resistance to this drug. Knowledge of biomarkers for miltefosine resistance may be beneficial for proper selection of treatment regimen.

Microarray-based gene expression profile of miltefosine relapsed parasites over sensitive strains

Differential gene expression of A. Calpain family cysteine protease-like protein and B. hypothetical protein 2 using qualitative real time RT-PCR

Cloning, expression and immunoblotting of recombinant Calpain family cysteine protease-like protein and hypothetical protein

Methods & Materials: Splenic aspirates were collected and parasites cultured from patients relapsed after initial cure (n=15) and successfully treated (n=15) with miltefosine. Differentially expressed genes in cultured parasite strains of miltefosine resistant strains, obtained by DNA microarray, were further validated by real time reverse transcriptase polymerase chain reaction (RT-PCR) and western blotting after preparing recombinant proteins.

Results: Out of 7705 gene specific probes labelled on a microarray chip, 669 genes were found to be up-regulated and 470 down regulated in resistant strains. The cysteine protease-like protein of calpain family [GenBank: CBZ34784] was found significantly over expressed in resistant parasite strains both by DNA microarray as well as real time RT-PCR. Only sera from relapse patients showed presence of anti calpain antibodies through western blotting.

Conclusion: Calpain family cysteine protease-like protein can be useful as potential biomarker of miltefosine unresponsiveness.
Endoscopic nodular gastritis with helicobacter pylori infection: An indicator of high-grade bacterial colonization and severe gastritis in children

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Background: Helicobacter pylori infection is a common and universally distributed bacterial infection. It is predominantly acquired in childhood. Endoscopic findings of antral nodularity can be seen in children much more frequently than in adults and believed that this gross change may suggest H. pylori infection and histologic gastritis. We conducted a study to assess the significance of Helicobacter pylori infection associated with endoscopic nodular gastritis (NG).

Methods & Materials: This prospective study carried out over two years period and included 468 children in whom upper digestive endoscopy was performed for gastrointestinal symptoms and gastric antral mucosal biopsy was taken. Sixty-seven children were diagnosed as having NG and were included in the study. Demographics, clinical characteristics, endoscopic and pathologic findings were recorded. H pylori were recognized in gastric biopsy on H&E sections; a modified Giemsa stain was performed in biopsy suspicious for H pylori.

Results: The prevalence of NG in children was 14.3% (67/468) and consisted of 46.3% male and 53.7% female. Children age ranged from 3 - 18 years (mean age, 9.2 ± 0.4 years). The prevalence of NG increased gradually with age. H pylori infection was identified in 68/468 (14.5%) children. Nodular gastritis had a poor accuracy rate to determine H. pylori infection (sensitivity, 40.3%; positive predictive value, 39.7%) and was observed in 27/68 (39.7%) H pylori positive patients and in 40/400 (10%) H pylori negative patients. There was a significant increase in grade of inflammation, activity, atrophy, number of lymphoid follicles and H. pylori density on histologic evaluation in H pylori positive patients with NG than other groups.

Conclusion: Nodular gastritis has a poor prediction for H. pylori infection in children. Gastric biopsies should always be obtained during endoscopy in children to establish the H pylori infection. H. pylori infection in children with NG identifies cases with severe gastritis and marked bacterial colonization.
Microarray-based assay for simultaneous identification and drug resistance detection of microorganisms causing sexually transmitted diseases

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Background: Sexually transmitted diseases (STDs) caused by microorganisms are among the most dangerous and rapidly spreading infections. STDs are often polymicrobial and caused not only by obligate pathogens, but also by a variety of conditionally pathogenic microorganisms, that may result in an atypical course of the diseases. The worldwide increase of drug-resistant STD's strains is also major challenge that necessitates the elucidation of the resistance mechanisms and development of new methods of genetic analysis suitable for implementation in clinical laboratory practice.

Methods & Materials: A molecular assay based on original technology of EIMB hydrogel microarrays has been developed for simultaneous identification and detection of drug resistance determinants of STDs causative agents. The microarray comprises immobilized oligonucleotides based on the STDs sequence-specific fragments of 16S rRNA loci and sequences of fragments of the rrl, gyrA, parC, mefA, mtrR, nimB-G, penA, ponA, porB, rpsJ, ntr4tv, ntr6tv, blaSHV, blaTEM, tetM, tetO genes - genetic markers of drug resistance. The procedure included multiplex amplification with simultaneous fluorescent labeling of PCR-products followed by hybridization on microarray. The assay was evaluated using 212 DNA samples isolated from clinical specimens containing STDs agents. Real-time PCR, target sequencing and partial conventional drug susceptibility testing were used as reference tests.

Results: A developed method provides identification of 17 different obligate and conditional pathogens including Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum, Mycoplasma genitalium, Atopobium vaginae, Bacteroides fragilis, Enterococcus faecalis, Escherichia coli, Fusobacterium nucleatum, Gardnerella vaginalis, Mobiluncus mulieris, Mycoplasma hominis, Staphylococcus epidermidis, Streptococcus anginosus, Trichomonas vaginalis, Ureaplasma parvum and Ureaplasma urealyticum with coincident analysis of 49 genetic determinants of resistance to macrolides, aminoglycosides, tetracyclines, aminocyclites, quinolones and nitroimidazoles. Sensitivity and specificity of the assay exceeded 95% for identification of STDs agents, and were 80 - 92% for detection of resistance to different antimicrobial drugs.

Conclusion: The proposed multiplex assay is a useful tool for selection of personalized treatment of STDs providing rapid and accurate identification of pathogens in clinical samples with simultaneous determination of resistance markers to a series of antimicrobial drugs. This work was financed by subsidy #14.607.21.0065 (RFMEFI60714X0065) from the Ministry of Science and Education of Russian Federation.
Temperature and oxidative stress as triggers for virulence gene expression in pathogenic Leptospira spp.

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**Background:** Leptospirosis is a zoonosis, widely distributed throughout the world and aetiology caused by pathogenic bacteria belonging to the genus, *Leptospira*. Environmental signals such as shifts from environmental (30°C) to physiological (37°C) temperatures or increases in oxidative stress can trigger response regulatory modes of the organism during the infection process. These responses might be mediated by upregulation, attenuation or silencing of genes whose expression is secondary to the organism’s survival. This study sought to determine the effect of temperature and oxidative stress on virulence associated genes in highly-passaged *L. borgpetersenii* Jules and *L. interrogans* Portlandvere.

**Methods & Materials:** Bacteria were grown in EMJH at 30°C, 37°C or at 30°C before being transferred to 37°C. A total of 16 virulence-associated genes (*lipL45, tlyC, Isa24, fliY, lfh, mce, lipL36, loa22, invA, ompL1, spH2, lipL32, hap1, Isa21/lenA, and lipL41*) were assessed using endpoint RT-PCR. To assess oxidative stress, bacteria were exposed to H₂O₂ for 30 and 60 min with or without the temperature stress.

**Results:** Transcriptional expression of virulence associated genes in *L. interrogans* Portlandvere (19%) was significantly lower when compared to expression in *L. borgpetersenii* Jules (57%). Only Jules expressed virulence genes at all temperatures. Genes upregulated following elevated temperature or temperature shift included *loa22* (in both), *hap1* and *lipL32* in Portlandvere and *invA* and *Isa21* in Jules. Genes expressed during oxidative stress included *loa22, lipL32* and *lipL41* in both serovars, and *fliY* and *Isa21* exclusively in Portlandvere.

**Conclusion:** While it is clear that expression of many virulence genes in highly passaged strains of *Leptospira* are attenuated or lost, the differences in gene expression between *L. interrogans* Portlandvere and *L. borgpetersenii* Jules may be attributed to the transmission cycle of the bacteria and/or regulation by other contributing factors. Overall, serovar Jules retained more potential for causing infection even though highly passaged.
Prevalence of virulence determinants among HA-MRSA and CA-MRSA isolates and pathogenicity testing using caenorhabditis elegans model

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Background: Methicillin-resistant Staphylococcus aureus (MRSA) has established itself as a major human pathogen causing both hospital and community acquired infections, especially due to its ability to express a myriad of virulence factors. There is paucity in data regarding the prevalence of virulence factors among MRSA, its role in pathogenesis and associated severity of infections from our part of the country. Hence, the present study has been designed to use C. elegans, a simple nematode model for pathogenicity testing and to demonstrate the virulence potential of HA & CA-MRSA.

Methods & Materials: A total of 100 S. aureus clinical isolates from community and hospital settings were included for the study. Methicillin resistance was screened using cefoxitin disc (30µg) and confirmed by a rapid triplex PCR for mecA, femA, pvl genes. Multiplex PCR was done to detect leukocidins (lukD, lukE, lukM), hemolysins (hla, hlb, hld, hlg) and 14 enterotoxins (sea-see, seg-seo). Nematode killing assay was performed by exposing stage-synchronized C. elegans L4-larvae to four representative HA-MRSA and CA-MRSA strains and phase-contrast microscopy was done to observe pathological effects.

Results: Of the 100 S. aureus isolates, 39 and 61 isolates were from hospital and community settings of which 64% were MRSA. 33/61 isolates (54%) from community settings were CA-MRSA and 31/39 (79%) isolates from hospitalised patients were HA-MRSA. Among the leukocidins tested, prevalence of pvl was highest (38%) followed by lukD and lukE in 32% of isolates. All isolates were positive for hla, hld; 6 harbored hlg and 1 isolate carried hlb; prevalence of sea, sec was predominant (16% and 7%). Nematode killing assay revealed that LD₅₀ of C. elegans L4-larvae ranged from 6-12hrs for HA-MRSA and 12-60hrs for CA-MRSA strains. Following severe intestinal infection and prolonged paralysis, complete killing of animals occurred between 66-114hrs.

Conclusion: The present study documents the prevalence of various virulence factors among MRSA isolates. This preliminary study using C. elegans model has indicated higher pathogenesis and rapid killing of HA-MRSA strains which showed higher prevalence of leukocidins (pvl, lukD, lukE) and enterotoxins (sea, sec) than CA-MRSA and so could serve as a model for pathogenesis testing for S. aureus.
Modeling of cerebral tuberculosis in BALB/c mice using clinical strain from patients with CNS -TB infection
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**Background:** This study describes development of a TBM animal model in mice using C3 strain isolated from CSF of TBM patients which can be used to study pathogenesis of C3 strain in mice.

**Methods & Materials:** Female BALB/c mice aged 6–10 weeks were housed in a BSL-3 facility. Two groups of mice (n=12) were challenged intravenously through tail vein with $2 \times 10^7$ of C3 strain. Control group of mice (n=10) was separately maintained and injected with sterile saline. Mice were killed at 30 and 50 days after development of infection, for estimating number of mycobacteria colonizing in brain, lungs and for histopathological changes. The mycobacterial burden was determined by plating and counting the number of CFUs. Serially diluted homogenates of individual lungs and brains were plated onto Middlebrook 7H11 medium. CFU’s were counted after 3-4 weeks of infection. Histological sections were stained using hematoxylin and eosin.

**Results:** Mice infected with C3 strain showed prominent edema of the brain in left hemisphere at 30 days, which increased at later stage compared to control mice. Histopathological examination of brain showed swelling of neurons along with lymphocytic infiltration which was progressively more at 50 days. The lung section at 30 days revealed >40 lesion in lung parenchyma which improved at 50 days, with lesion reduced to <20. Brain and lungs of control mice showed no major changes. Infection with C3 strain showed significant levels of mycobacterial load in brains with progress in infection. Analysis of CFU count revealed significantly high load in lungs (6.80±0.1) at 30 days post infection, however the mycobacterial burden gradually decreased in lungs (5.8±0.2) with progress in infection at 50 days. Analysis of CFU load in brain showed significantly high mycobacterial load at 50 days (4.0±0.2) compared to infection at 30 days (5.0±0.1). No mycobacterial load was observed in brains and lungs of control mice. C3 strain infection was associated with reduced survival-40% and high mortality rate -60% compared to control mice.

**Conclusion:** Our present study demonstrated that intravenous inoculation by C3 strain from TBM patient’s leads to progressive dissemination and development of TBM disease in mice.
Genome wide differential host response to highly or low pathogenic H5N1 avian influenza virus infection in ducks
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Background: The underlying molecular mechanisms of pathogenesis and outcome of disease to different pathotypes of H5N1 influenza infection in ducks remain unclear.

Methods & Materials: Hence, we studied genome wide host gene expression of duck lung tissues infected with A/duck/India/02CA10/2011 (AD2011) and A/duck/Tripura/103597/2008 (AD2008) H5N1 viruses using custom designed microarray. AD2011 is highly pathogenic whereas AD2008 is low pathogenic to ducks.

Results: Comparative analysis of differentially expressed genes revealed that 688 genes were commonly expressed, 877 and 1556 genes are uniquely expressed to infection with AD2011 and AD2008 virus isolate, respectively. The up-regulation of cytokine (IL17), chemokines (CCL4 and CXCR4) and IFN stimulated genes (OAS, IFITM2, STAT3, TGFB1 and TGFB2) in the lungs tissues possibly caused high mortality in ducks infected with AD2011 virus. The expression of important antiviral immune genes IFIT5, IFITM5, RSAD2, EIF2AK2, Mx, β-defensins, TRIM23 and SLC16A3 in AD2008 infection, but not in AD2011 infection, might fine-tune the innate immune responses and prevent cytokine storms and tissue damage. Several immune related gene ontology terms and pathways activated by both the viruses were qualitatively similar but quantitatively different.

Conclusion: Based on these findings, we conclude that subtle differences in host immune responses may determine the different outcome of H5N1 infection in ducks.
Differential dendritic cells responses to infection with various serotypes of shigella

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**Background:** Dendritic cells (DC) are key regulators of immune response with the ability to affect both the innate and adaptive immune responses and are abundant in the gut mucosa. The severity of shigellosis varies with the serotype involved with *S. dysenteriae* (SD) producing the severest infections and complications with *S. sonnei* (SS) being at other end of spectrum usually causing mild self-limiting diarrhea. While shigellae are known to induce the apoptosis of mature DCs, there is no information in cytokine milieu of DCs incubated with different serotypes of *Shigella*.

**Methods & Materials:** Monocyte derived dendritic cells (MoDCs) were developed from healthy human PBMC after 8 days of culture. They were characterized by four-color flow cytometry technique using Becton Dickinson FACS ARIA III, equipped with 488 nm and 630 nm argon laser and analysed by FACS Diva 6.1.2 Software on the basis of CD11c positive, HLA-DR positive and CD3 negative. DCs were infected with different Shigella serotypes. After 24 hour post infection, relative expression of cytokines IL-1β, IL-6, IL-8, TNF-α, IL-12p70, IL-17, IL-22 and IL-23 was studied by Real Time PCR and data was analysed by Graphpad prism 5.

**Results:** IL-8, IL-17A, IL-22A and IL-23 expressions were highest in MoDCs stimulated with *S. dysenteriae* serotype1 and significant serotypic differences were noted between SD & SF and between SD & SS. The transcription levels of IL-23 were down regulated in *S. flexneri* & *S. sonnei* in comparison to normal MoDCs. IL-8 appears to be a major molecule orchestrating mucosal inflammation in shigellosis. It is the primary cytokine which induces neutrophil chemotaxis. SD1 induces more Th17 response which displays pro-inflammatory functions. IL23 is responsible for the expansion of Th17 previously differentiated. IL-23 promotes the development and expansion of activated CD4+ T cells.

**Conclusion:** DCs are critical sentinel cells that relay microbial presence either directly or indirectly to naive T cells. In this study we found that *Shigella dysenteriae* caused maximum release of IL-8. Similarly SD also caused highest release of IL-17A and IL-22A. It was the only serotype which increased IL-23. These findings could explain more severity of SD as compared to SF and SS.
Genome wide host gene expression analysis in chicken lungs infected with avian influenza viruses
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Background: The molecular pathogenesis of avian influenza viruses vary greatly with individual bird species and virus strain. The molecular pathogenesis of the highly pathogenic avian influenza virus (HPAIV) or the low pathogenic avian influenza virus (LPAIV) in avian species remains poorly understood.

Methods & Materials: Thus, global immune response of chickens infected with HPAI H5N1 (A/duck/India/02CA10/2011) and LPAI H9N2 (A/duck/India/249800/2010) viruses was studied using microarray to identify crucial host genetic components responsive to these infection.

Results: HPAI H5N1 virus induced excessive mRNA expression of type I IFNs (IFNA and IFNG), cytokines (IL1B, IL18, IL22, IL13, and IL12B), chemokines (CCL4, CCL19, CCL10, and CX3CL1) and IFN stimulated genes (OASL, MX1, RSAD2, IFITM5, IFIT5, GBP 1, and EIF2AK) in lung tissues. This dysregulation of host innate antiviral genes may be the critical determinant of the severity and the outcome of the influenza infection in chickens. In contrast, the expression levels of most of these genes remained unchanged in the lungs of LPAI H9N2 virus infected chickens.

Conclusion: This study indicated the relationship between host antiviral genes and their roles in pathogenesis of HPAIV infection in chickens.
Murine model of tuberculous meningitis: New insight into understanding pathological complications of the disease

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Background: Tuberculous meningitis is most severe presentation of tuberculosis that causes mortality in one-third of the affected patients. Though an age old disease; it still remains an inefficiently treated infection of human brain. Main reason behind is less known pathophysiology of the disease which is due to limited studies in developing proper experimental model.

Methods & Materials: Mice model of tuberculous meningitis which mimics the symptoms of human disease was developed in this study. A burr hole of 1.5mm in diameter was made at the inoculation site after midline sagittal incision. Mycobacterium tuberculosis H37Rv infection (10⁵ CFU/50µl) was intracranially inoculated and scalp incision was stitched aseptically. After 4, 8 and 12 weeks of infection, mice were sacrificed and tissues processed for CFU enumeration, histopathology and analysis of inflammatory marker (MMP-9).

Results: Bacillary load in tissues was stable and showed gradual increase from 4 to 12 weeks of infection. In brain and lungs, the increase in CFU counts was found to be significantly high after 12 weeks of infection. Histopathological examination of mice brain showed apparent meningitis on surface of brain, denser meningitis and edema and heavy meningitis with extensive edema at 4, 8 and 12 weeks post infection respectively. Mice lungs showed lymphocytic cuffing around granuloma, presence of lymphocytic granuloma and consolidation due to tuberculous pneumonia at 4, 8 and 12 weeks post-infection respectively. Besides, dissemination of bacilli from brain to other body parts was confirmed by presence of bacilli in lungs by AFB staining. TB pathology in spleen was confirmed by generation of mild reactions at 4th week of infection and non-follicular reaction at 12th week postinfection. Analysis of MMP-9 showed inflammatory response to be gradually increasing after 8 weeks of infection. Neuromuscular coordination of mice as monitored using Rota rod test was affected after 8 and 12 weeks of infection.

Conclusion: Tuberculous meningitis developed in this study highlights histopathological changes and locomotory disability which can be extrapolated to the human disease. Therefore it can be used as a suitable model to study various aspects of the disease and also evaluate newer drugs and therapeutic targets for efficient treatment of the disease.
Background: Infections with Methicillin resistant \textit{Staphylococcus aureus} (MRSA) have become a major challenge to healthcare delivery, because of the difficulty in treating them. Carriage of this multidrug resistant organism by individuals has been the means by which it persists in the environment. Healthcare workers tend to become colonized due to their close contact to patients and poor adherence to infection control. The aim of this study was to determine the nasal carriage of MRSA among healthcare workers in high risk units of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria and the antibiotic susceptibility pattern of the isolates.

Methods & Materials: This descriptive cross-sectional study was carried out between January to November 2014. Using stratified random sampling among different categories of healthcare workers, nasal swabs were collected, screened for MRSA using cefoxitin disk diffusion, then confirmed by testing for the \textit{mecA} gene product using latex agglutination test for PBP2a.

Results: From the 427 health workers swabbed, 81 (19\%) \textit{S. aureus} isolates were identified using Staphaurex®\textsuperscript{a}, among which 10 (12.3\%) screened MRSA positive using cefoxitin disk, all of which were confirmed by Oxoid latex agglutination test for PBP2a to be MRSA, giving an overall nasal carriage of 2.3\% for MRSA from the total population studied. Carriage was found mainly among nurses (60\%) and doctors (40\%), with the highest proportions from oncology (50\%) and orthopaedic (25\%) units. There was no significant difference in carriage between the ages (P=0.702), and length of stay in the various high risk units (P=0.89). The highest resistance rates were to penicillin (90.1\%), then sulphamethoxazole-trimethoprim (28.4\%), however, there was almost universal (98.8\%) susceptibility to gentamicin and complete susceptibility to rifampicin (100\%). Most (60\%) of the MRSA isolates were however multidrug resistant.

Conclusion: The carriage from this study is low compared to what has been reported from other parts of Nigeria. The pattern of resistance to other antibiotics tally with reports from other centers in Africa. Continuous vigilance, improved infection control practices and antibiotic stewardship program is necessary to maintain this low prevalence.
Antibiotic sensitivity and resistance patterns of salmonella typhi isolates from Nigerian malnourished children

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**Background:** Malnourished children are at an increased risk of infection especially Gram Negative septicaemia. Gram negative septicaemia especially caused by *Salmonella typhi* in malnourished children is associated with increased mortality. Appropriate knowledge of the local antibiotic susceptibility pattern will lead to optimal choice of antibiotic treatment hence reducing its associated mortality. This study aimed at determining the local antibiotic susceptibility and resistant patterns of the *Salmonella typhi* isolates from malnourished children.

**Methods & Materials:** We analysed susceptibility data for all *Salmonella typhi* isolated from the blood culture of malnourished children in Aminu Kano Teaching Hospital Kano Nigeria from May to October 2013. Antimicrobial susceptibility assessment was performed on all bacterial isolates using Kirby-Bauer disk diffusion method for locally available antibiotics. The susceptibility testing was based on the standards published by the clinical laboratory standard institute (CLSI).

**Results:** There were seven *Salmonella typhi* isolated from the 41 bacteraemic malnourished children. All the isolates were reported as susceptible to Ciprofloxacin, Ofloxacin and Erythromycin. Eighty percent were susceptible to Ceftriaxone and Gentamicin. All the isolates were resistant to Cloxacillin, Co-Trimoxazole, Amoxicillin and Augmentin.

**Conclusion:** These data showed resistance of the isolates to the antimicrobial recommended by the world health organization in the treatment of malnourished children with suspected bacterial infection, hence calls for its revision.
Comparison of bacterial characteristics (MICs) of Gram negative bacteria isolated from patients with neutropenic sepsis pre and post-Levofloxacin prophylaxis

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Background: Febrile neutropenia is a life-threatening complication, that occurs frequently during chemotherapy with associated high mortality. Antibacterial prophylaxis is an established strategy to prevent this. Fluoroquinolone prophylaxis has been considered for high-risk patients with prolonged and profound neutropenia (ANC<1000mm³), but risk of emergence of resistance has been a concern. Levofloxacin was used as prophylaxis during the neutropenic period in chemotherapy-induced neutropenic patients at Leicester Royal Infirmary Hospital(LRIH), United Kingdom since 2010.

Methods & Materials: Compare number of blood culture positivity in pre(2007-2010) and post(2010-2012) Levofloxacin prophylaxis periods and compare sensitivity of Ciprofloxacin and Meropenem in Gram negative isolates from neutropenic patients of above periods in LRIH.

Design, setting and methods: Retrospective data collection done using haematology Gram negative bacteraemia data base, and relevant clinical and laboratory data were retrieved from case notes and computer based data system. VITEC-MIC and E-strip MIC for Ciprofloxacin & Meropenem were performed on the Gram negatives retrieved from saved beads. From 210 total blood culture positives of pre-levofloxacin period, 45 isolates for ciprofloxacin MIC and 44 isolates for meropenem MIC performed. From 88 total blood culture positives of post-levofloxacin period, 79 for ciprofloxacin MIC and 78 isolates for meropenem MIC performed.

Results: Number of blood culture positivity has reduced from 210 to 88 with prophylaxis. Both MIC methods (VITEC & E-strip) given similar sensitivities for tested Gram negative isolates. Resistant rate for Meropenem was 4.5% (2/44) in pre-prophylaxis period and 11.5% (9/78) in post-prophylactic period. Resistance to Ciprofloxacin was 17.7% (8/45) in the pre-prophylaxis period and 25% (20/79) in the post prophylaxis period. Both differences were not statistically significant at a p value of 0.05 (Fisher's exact test)

Conclusion: The use of Levofloxacin as a prophylactic agent had not resulted in a statistically significant increase of resistance among the common Gram negative pathogens. However, close monitoring is warranted as a trend towards increase in the proportion of resistance was noted.
Prevalence and antibiotic sensitivity profiles of bacteria causing community acquired pneumonia

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Background: Despite the availability of potent new anti-microbial agents and vaccines, Community Acquired Pneumonia (CAP) remains a common and serious illness. The microbial etiologies and their resistant patterns vary widely. Frequent, irrational and unnecessary use of antibiotics, changes in environment, changes in lifestyle and increased mobility of the people have contributed to changes in the patterns of microbial profiles and their resistant patterns in the Community. Unfortunately, there have been very few studies done regarding etiology of CAP, prevalence of causative organisms and their resistance pattern in Nepal. Thus it was quite crucial to detect them in our context

Methods & Materials: A descriptive cross-sectional study conducted over a period of six months (March 2011-August 2011) at Bacteriology laboratory of Tribhuvan University and Teaching Hospital (TUTH) among 600 clinically diagnosed CAP patients visiting TUTH-OPD. Sputum samples that met the acceptance criteria of ASM were further processed according to the standard methodology.

Results: Bacterial etiologies could be identified only in 25.5% of cases of CAP. Haemophilus influenzae (26.9%), Streptococcus pneumoniae (20.0%) and Pseudomonas aeruginosa (19.4%) were the commonest bacterial etiologies. Twenty-six percent of H. influenzae isolates were MDR. The prevalence of MDR bacteria in CAP patients was 41.25%. Among gram-negative bacterial isolates, the highest number of MDR was seen in Pseudomonas aeruginosa, followed by Klebsiella pneumoniae, E. coli and Acinetobacter spp. The prevalence of ESBL, AmpC and MBL producing gram-negative bacteria were 10.1% (more common among Klebsiella pneumoniae (16.6%), 5.8% and 4.3% (more common among Acinetobacter spp(14.29%)) respectively.

Conclusion: Different bacteria are responsible for CAP in our setting. MDR, ESBL, MBL and AmpC producing bacterial strains are present in our Community also. Thus it has demanded to take special care during treatment of patients with community acquired infections also and also sought for other similar type of extensive studies on large number of community isolates to characterize their genetic relatedness and resistant patterns so that appropriate measures can be applied.
Antibiotic resistance pattern of HA-MRSA strains isolated from leukemia patients in Baghdad, Iraq

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**Background:** *Staphylococcus aureus* (*S. aureus*) is an important pathogen for hospital-associated infections, and it is especially difficult to treat because of emerging resistance to many antimicrobial drugs. Methicillin-resistant *S. aureus* (MRSA) have emerged as a clinically relevant human pathogen. Intracellular destruction of invading *S. aureus* is mainly mediated by phagocytosis, and this phagocytic process is crucial to the host innate defense against Staphylococci, so, disorders of phagocytic cells- as in cases of leukemia- cause frequency of infections at epithelial surfaces and the frequency of dissemination. The aim of study is to evaluate MRSA strains isolated from leukemia patients admitted to hospitals in regard to drug resistance.

**Methods & Materials:** A cross-sectional study was conducted at the Hematology center/ Al-Mustansiriya University- Baghdad, from November 2013 to March 2014, where 48 known leukemia patients aged from 15 to 81 years have been enrolled for the detection of MRSA in their blood. Blood culture and conventional methods were used for the isolation and identification of pathogenic bacteria, along with the phenotypic and genotypic methods (i.e. detection of “PBP2a”) for the identification of MRSA. Antibiotic susceptibility testing and vancomycin minimum inhibitory concentration (MIC) were also performed for all *S. aureus* isolates.

**Results:** Positive blood culture results were detected in 34 (71%) of patients. Among them, *S. aureus* was accounted for 11 (26.8%) of isolates. Antibiotics susceptibility tests for *S. aureus* isolates showed that 9 (82%) strains isolated from patients in current study were of MRSA variety. All of the *S. aureus* strains (100%) in current study were susceptible to Carbapenems. Intermediate resistance to vancomycin was noticed in 4 (36.4%) *S. aureus* isolates (i.e. VISA strains), with no totally vancomycin-resistant (VRSA) strains. All of phenotypic MRSA isolates, except of one, were positive for PBP2a.

**Conclusion:** The study concludes that *S. aureus* is the most prevalent pathogen causing bacteremia in leukemia patients, with MRSA variety comprising the majority of these strains. Also, phenotypic method for MRSA detection can be performed using either of Methicillin, Oxacillin, or Cefoxitin with same results, with a non-significant statistical difference between the phenotypic method and the genotypic method- via the PBP2a detection.
Prevalence and risk factors for intestinal colonization with vancomycin resistant enterococci among patients admitted to intensive care units of a large teaching hospital in Southern India

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Background: Vancomycin Resistant Enterococci (VRE) emerged as one of the major nosocomial pathogen across the globe. Gut colonisation rate with VRE is higher in patients admitted to ICUs due to high antibiotic pressure. VRE colonisation increases the risk of developing infection up to 5-10 folds. The aim of this study was to determine the rates of VRE colonization among patients admitted in two of the ICUs i.e. Medical Intensive Care Unit (MICU) and Pediatric Intensive Care Unit (PICU) and to assess the various risk factors which are associated with VRE colonization.

Methods & Materials: Rectal swabs were collected after 48 hours of ICU admission from a total of 302 and 198 patients from MICU and PICU respectively. Additionally samples were collected every 48 hours from 32 patients admitted in MICU and 19 patients in PICU whose initial VRE colonization was negative. The samples were inoculated on to Bile Esculin Sodium azide Agar (BEA) with 6mg/ml of vancomycin. Growth on this medium were identified by standard biochemical test and Minimum Inhibitory Concentration (MIC) of vancomycin and teicoplanin was detected by Agar dilution method. Resistance genes for vancomycin were detected by PCR. Risk factors were assessed by logistic regression analysis. The patients were followed up to determine VRE infection rates

Results: The rates of VRE colonization in patients admitted to MICU was significantly higher (29%) than those in PICU (19 %). Majority of the isolates were Enterococcus faecalis (62.6%) followed by Enterococcus faecium (38.4%). All the VRE isolates were positive for vanA gene. Younger age, increased duration of hospital stay, consumption of ceftriaxone and vancomycin were found to be significantly associated with VRE colonization in MICU, while in PICU, only vancomycin usage was the significant risk factor. Among VRE colonized patients, six (4.7%) acquired VRE infection.

Conclusion: The VRE colonization rates in our ICUs were comparable to other hospitals worldwide. Strict adherence to hand hygiene and education of health care workers is necessary to minimize the nosocomial transmission of this organism.
A study of 24 patients with colistin resistant gram negative isolates in a tertiary care hospital in South India

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**Background:** As the use of colistin to treat carbapenem resistant gram negative infections increases, colistin resistance is being increasingly reported in Indian hospitals.

**Methods & Materials:** Retrospective chart review of clinical data from patients with colistin resistant isolates (MIC >2). Clinical profile, outcome and antibiotic combinations that were used to treat colistin resistant infection were analysed.

**Results:** Twenty four colistin resistant isolates were reported over 18 months (Jan 2014–June 2015). The mean age of the patients was 58.33; average length of stay was 36.37 days. Previous hospitalisation within 3 months was noted in all 24 patients. An invasive device was used in 22 (91.67%) patients. Urine was the commonest site of infection=8(33%), followed by blood= 6(25%), respiratory=5(20.8%), pus=4(16.67%) and other (CSF)=1(4.17%). Commonest organism was Klebsiella, n=21(87.5%). Antibiotics that were used in their current admission prior to isolating a colistin resistant organism were: colistin in 15 patients(62.5%), carbapenem in 19(79.17%), BL-BLI in 9(37.5%), tigecycline in 12(50%). 16(66.6%) were considered to have true infection while 8 (33.3%) were considered as colonisation and were not treated. Sensitivity of these isolates to other drugs tested were – tigecycline 18/24(75%), chloramphenicol 15/24(62.5%), amikacin 7/24(29.17%), cotrimoxazole 3/24(12.5%). Fosfomycin was tested in 4 isolates only and was sensitive in all. Antibiotic that were used for treatment were combinations of tigecycline, chloramphenicol, fosfomycin, amikacin, ciprofloxacin, cotrimoxazole and sulbactam. Among 16 patients with true infection, 4(25%) improved, 9(56.25 %) expired and 3(18.75%) were transferred to other centers in poor condition at patient's request. Among 8 patients who were considered to have colonisation, there were no deaths and 7(87.5%) improved. Among the 6 bacteremic cases, 5 patients expired and 1 improved. Among the non-bacteremic patients with true infections 4/10(40%) expired. Bacteremic patients had a significantly higher risk of death compared to all non-bacteremic patients (p=0.014) though not significantly in non-bacteremic infections after exclusion of colonization (p=0.145).

**Conclusion:** Colistin resistance among Gram negative bacteria, especially Klebsiella, is emerging in Indian hospitals and carries a high crude mortality. Prolonged hospitalization (36 days on average), use of invasive devices and prior colistin exposure may be risk factors. Tigecycline, chloramphenicol and fosfomycin may be options for combination therapy.
Genotypic and phenotypic characterization of antimicrobial resistance in staphylococcus aureus strains isolated from wound infections in Mardin, Southeastern Turkey

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Background: Nosocomial and community-acquired infections caused by Staphylococcus aureus are still a worldwide growing problem. The aim of this study was to evaluate the phenotypic and genotypic antimicrobial susceptibility patterns of S. aureus isolated from wound samples of Turkish patients living in Mardin, Southeastern Turkey.

Methods & Materials: A total of 220 clinical wound samples, collected between December 2012 - August 2013 were used in this study. The identification of S. aureus was made by conventional methods. The antimicrobial susceptibility of S. aureus strains to eleven antimicrobial agents was performed by the disk diffusion method and the results were evaluated according to EUCAST 2014 guideline. Additionally, DNA was extracted from the wound samples using the High Pure PCR template preparation kit. Eleven genes indicating the genotypic resistance to oxacillin (mecA), gentamicin (aac(6′)-aph(2′), aph(3′)-IIIa, ant(4′)-Ia), erythromycin (ermA, ermB, ermC, and msrA), tetracycline (tetK, tetM) and penicillin (blaZ) were amplified using multiplex PCR and were visualized using gel imaging.

Results: S. aureus was isolated from 112 (50,91%) of 220 wound samples. The phenotypic resistance rates of S. aureus was found 45.54% (51 strains) for penicillin G, 41.07% (46 strains) for ampicillin, 33.04% (37 strains) for tetracycline, 26.79% (30 strains) for erythromycin, 5.36% (6 strains) for trimethoprim-sulfamethaxasol, 4.46% (5 strains) for oxacilline, 0.89% (1 strain) for amoxycillin-clavulanic acid and 0.89% (1 strain) for enrofloxacin. All isolates were found to be susceptible to gentamicin, vancomycin and teicoplanin. None of isolates showed an aminoglycoside resistance genotypically. The number and rates of MSSA and MRSA carrying antibiotic resistance genes were 19 (16,96%) and 32 (28,57%) for blaZ, 9 (8,04%) and 17 (15,18%) for tetK, 6 (5,36%) and 14 (12,5%) for ermC, 2 (1,79%) and 7 (6,25%) for tetM, 0 (0%) and 5 (4,46%) for mecA, 2 (1,79%) and 4 (3,57%) for ermA, 1 (0,89%) and 2 (1,79%) for both tetK and tetM respectively.

Conclusion: The data obtained facilitate the nationwide surveillance of the antimicrobial resistance gene profiles of S. aureus for accurate treatment of patients and to control the dissemination of resistance genes.
Frequent resistant gram negative rod stool colonization among patients admitted with acute febrile illness in Pune, India

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Background: Antimicrobial resistance is increasing worldwide, including in India. There is a concern that unfettered antibiotic use may be associated with increasing antimicrobial resistance. Surveillance for antimicrobial resistance may be accomplished by evaluating for stool colonization of drug resistant organisms.

Methods & Materials: Patients > 12 years of age admitted to adult medicine wards at BJ Medical College - Sassoon General Hospital, in Pune, India with > 1 day of fever were enrolled into a prospective cohort between July 2013 and September 2015. A perianal swab sample was collected on the day of enrollment and on day 3-5 of hospitalization and stored at -80°C pending processing. Samples were aspirated onto peptone broth impregnated with ceftriaxone and vancomycin, incubated, and then plated onto MacConkey and blood agar. Identification and drug susceptibility testing was performed on isolates using a Phoenix system (Becton Dickinson).

Results: Perianal swabs were collected on 314 patients at the time of enrollment. Follow up swabs were collected on 181 patients – 158 on day 3, 22 on day 4, and 1 on day 5. There was growth of resistant gram negative rods (GNR) in 60 (19%) patients. In 9 (2.9%) of patients, there was growth of GNR resistant to imipenem. Seventeen patients who were not colonized with resistant GNR upon enrollment were colonized at the time of follow up. Inpatient use of cephalosporins was associated with acquisition of colonization with resistant GNR, odds ratio 3.68, 95% confidence interval 1.02-13.31.

Conclusion: Perianal colonization with resistant gram negative rods is common among patients admitted to medicine wards in Pune, India. The association of acquisition of drug resistance during a brief hospitalization with prescription of cephalosporins highlights the need for improved antimicrobial stewardship.
Molecular characterization of escherichia coli isolated from hospital acquired infections from two different geographical areas, Ujjain and Bangalore

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**Background:** Background
Health-care associated infections (HAI) caused by *Escherichia coli* have become a major challenge for clinicians due to their high frequency and changing epidemiology. Infections caused by ESBL producing *E. coli* are of particular concern. This study was conducted to identify the presence of genes coding for cephalosporin and quinolone resistance, integrons, phylogenetic groups and O25bST131 group among *E. coli* associated with HAI from two hospitals in Bangalore and Ujjain, India

**Methods & Materials:** Methods and Materials
The study was a cross-sectional laboratory based observational study from 2011 to 2012 was done in two different hospitals. A total of 303 and 212 *E. coli* isolates from 2011-2012, from St. John’s Medical College Hospital, Bangalore and the hospital associated with R D Gardi Medical College, Ujjain respectively, were included. In biochemically confirmed *E. coli* isolates, resistance to cephahlosporins and quinolones was determined by disc diffusion assay and further confirmed by agar MICs. ESBL were detected by combined disc diffusion method. ESBL coding genes, *bla* CTX-M, *bla* SHV, *bla* TEM ,*bla* OXA plasmid ampCand PMQR coding genes *qnr*A, *qnr*B, *qnr*S, oqxÅB, qepA,Class 1 (Intl 1) and Class 2(intl2) integrons, phylogenetic grouping and O25b-ST131 grouping was detected by PCR.

**Results:** Results
The majority (>90%) of *E. coli* isolates were resistant to both cephalosporins and quinolones. Among these 53% (n=162) and 65% (n=138) were multi drug resistant (MDR). MIC$_{90}$ for both, cefotaxime and ciprofloxacin was 512 µg/ml. CTX-M–group 1 coding genes were detected in 20% and 21%, whereas, plamid *amp*C genes were found in 3% and 12% isolates; The *qnr* B coding genes were detected in 7% and 12% while 72% and 51% of Class- 1 integron genes were present among *E. coli* isolates from Bangalore and Ujjain respectively. Phylogenetic group D was predominant. A total of 33% isolates from Bangalore and 28% from Ujjain belonged to O25bST131 group.

**Conclusion:** Conclusion
The increasing antibiotic resistance leading to increase in morbidity and mortality associated with HAI's emphasizes the need for molecular approaches in the diagnosis and epidemiologic analysis of HAI.
Transcriptional response of AcrAB-TolC conferring carbapenems resistance within escherichia coli associated with community acquired infection

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Background: Escherichia coli, an important member of Enterobacteriaceae is responsible for both hospital and community acquired infections. They have developed different mechanisms for survival in antibiotic stress. One of the well known mechanisms of resistance is the Efflux pump activity. These efflux pumps have the ability to export a wide range of compounds like antibiotics, detergents, organic solvents etc. A major tripartite multidrug efflux pump of Escherichia coli, AcrAB–TolC, confers resistance to a wide variety of compounds. The present study was undertaken to screen phenotypically efflux pump mediated carbapenems resistance by using an inhibitory based method in E.coli associated with community acquired infection.

Methods & Materials: A total of 293 E.coli samples were collected from Silchar Medical College and Hospital and Private Diagnostic Laboratories of Silchar Town isolated from clinical specimens. The samples were subjected to screening for efflux pump activity using carbonyl cyanide m-chlorophenylhydrazone as inhibitor in combination with meropenem. Antibiotic susceptibility testing was done by disc diffusion and MIC determination. Quantitative real time PCR was carried out to estimate the relative copy number of AcrA and the fold change was normalized against an endogenous control \textit{rpseL}. Relative quantities of gene expression were calculated using the $\Delta\Delta$Ct method.

Results: 103 isolates were found to be positive for showing Efflux pump mediated carbapenem resistance. The samples were tested for their antibiotic profiling and moderate susceptibility was observed with Amikacin (26%), Cefepime (22%), Gentamicin (20%), Co-Trimoxazole(19%), Aztreonam(18%), Piperacillin/Tazobactum (16%), Ceftriaxone (12%), Levofloxacin(10%), Ampicillin(8%), Ciprofloxacin(7%). RNA was isolated by using RNeasy Kit (Qiagen, India) from 35 samples having higher MIC of meropenem. Thereafter, cDNA was prepared from mRNA using QuantiTect Reverse Transcription Kit (Qiagen, India). All the Prepared cDNA were subjected to semiquantitative PCR. On the basis of semiquantitative PCR 21 samples were selected for qRT-PCR. Upregulation was observed in 11 samples and the rest 10 samples showed downregulation.

Conclusion: This study could establish the intrinsic resistance mechanism plays a significant role in carbapenem resistance in Escherichia coli. At the same time this study could help to understand the transcriptional regulatory mechanism underlying among carbapenem resistant Escherichia coli isolates.
Background: Antibiotic resistance is increasing worldwide, and is associated with severe morbidity, mortality and increased healthcare related costs. The frequency of extended spectrum beta-lactamase Enterobacteriaceae (ESBLE) is steadily increasing since the early 1980s. In Cambodia there are only few data published on the antimicrobial resistance even though antibiotics are not usually used appropriately in this country. The aim of this study is to evaluate the extension of ESBLE for a seven-year period.

Methods & Materials: Phenotypic methods done by disk diffusion are useful screen for detecting resistance profiles. The ESBLs detection is performed according to the French Society for Microbiology guidelines, with the disk synergy method: cefotaxim (30µg), ceftazidim (30µg), cefepim (30 µg), aztreonam (30µg) disks (Bio-Rad) placed by an automatic disk dispenser on Mueller-Hinton agar at a distance of 30mm, center to center, from an clavulanate disk (ticarcillin-clavulanic acid 10µg). The presence of ESBL was inferred when the inhibition zone around any of the five antibiotic disks was enhanced on the side of the disk containing clavulanic acid, resulting in a characteristically shaped zone referred to as a "champagne-cork" or "keyhole". Suspicion of carbapenemase production is based on the decrease of sensitivity to ertapenem, MIC ertapenem ≥ 0,5 mg/L or diameter < 28 mm and/or if there is at least one resistance to 3CG. Confirmation is done by using molecular biology kit using Real time PCR for detecting resistance genes: KPC, NDM, VIM and OXA-48.

Results: E. coli et K. pneumoniae are the most frequently encountered Enterobacteriaceae (E. coli: 61.25 %; K. pneumoniae: 19.88 %). Phenotypic screening showed resistance to carbapenems for 21 strains with MICs for ertapenem > 0,5mg/L, but only 10 were confirmed producing carbapenemase: 9 strains producing NDM-VIM, class B; 1 producing OXA-48, class D. ESBL enterobacteriaceae essentially circulate in community, 100% of secreting strains carbapenemase come from hospitals.

Conclusion: Both emergence of ESBL E.coli in the community and carbapenemases is a worrying phenomenon. For carbapenemase the most important risk is the secondary spread in the community, making them impossible to control and leading to therapeutic impasses. ESBL E.coli today represents a new type of faecal peril.
Expansion of diverse inc F type plasmids within enterobacteriaceae conferring multidrug resistant trait in tertiary referral hospital in north east India

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Background: Horizontal gene transfer is a common phenomenon for the dissemination of antibiotic resistance within a specific level. Acquisition of a resistant plasmid by a recipient organism depends on the incompatibility types that encode the resistance determinants. The present study characterizes incompatibility of resistant plasmids within enterobacteriaceae family and their transmission dynamics.

Methods & Materials: A total of 177 consecutive, non duplicate, gram negative enterobacteriaceae isolates were collected from indoor and outdoor patients of a tertiary referral hospital of north east India from a period of six months from September 2013 to February 2014. All the isolates were screened for multidrug resistant trait against beta lactam, aminoglycoside, quinolone group of antibiotics. Plasmids were isolated from the isolates showing multidrug resistance and subjected for transformation assay in different screen agar (gentamicin (0.25 µg/ml), ampicillin (100 µg/ml) and ciprofloxacin (0.25 µg/ml)) containing plates. Transformants were subjected for incompatibility determination by PCR based replicon typing.

Results: All the number of 177 isolates multidrug resistance against beta lactam, aminoglycoside and quinolones. Transformants of different resistant plasmids retained multidrug resistant phenotype in 163 isolates. On PCR based incompatibility typing it was found that F inc type was more predominant than other inc types F (n=35), followed by N (n=16), L (n=16), K/B (n=15). Among F types FIA (n=11), FIB (n=7), FIC (n=9), FrepB (n=8) was characterized.

Conclusion: This was probably the first study from this region typing plasmids based on their resistance profile. This study was indicative of diverse source and acquisition of resistant plasmid within hospital isolates in this region.
Bacteriological profile of blood culture isolates in a cancer hospital with special reference to E. coli and its Antibiotic susceptibility pattern in patients with Haematological malignancies

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Background:
Blood stream infections are an important cause of morbidity and mortality especially in febrile neutropenic patients. Escherichia coli (E. coli) is a pathogen of great concern in immunosuppressed patients. On time diagnosis and appropriate medication will be the best way to save the lives of the affected ones.

Methods & Materials:
The objective of the study is to describe the pattern of bacterial isolates from Blood cultures with special reference to E. coli as an important pathogen and to determine the antibiotic susceptibility pattern of E. coli. This is a retrospective analysis of 1455 Blood cultures collected from clinically suspected cases of bacteremia between Jan 2014-Oct 2015 in a cancer hospital. The isolates were identified by standard biochemical tests and also by fully Automated BD phoenix instrument. Antimicrobial susceptibility was done by the Kirby bauer disk diffusion method following the standard CLSI guidelines.

Results:
Positive cultures were obtained in 376 (25.84%) cases. Among culture positive isolates, Gram negative bacteria accounted for 51.59%, most common being E. coli (25.79%) followed by pseudomonas species (7.71%). Out of 97 non-repetitive E. coli isolated 76.2% were sensitive to Amikacin, 79.38% were sensitive to Imipenem, 45.36% were sensitive to Piperacillin + Tazobactam & 46.39% were sensitive to Cefaperazone + Sulbactam. 87.63% of E. coli isolates were resistant to Levofloxacin and Cefepime & 74.23% were resistant to trimethoprim and sulphamethoxazole.

Conclusion:
E. coli is a major pathogen in hematologic malignancy patients with neutropenia. As part of their treatment regimens, such patients are exposed to chemotherapy, radiation, and antimicrobials in a short time period. As a result, the equilibrium between the intestinal microbiota and mucosal epithelium is disrupted, causing large shifts in bacterial populations inhabiting the gut thus making the patient susceptible to bloodstream infections. Fluoroquinolones, are widely used to protect these patients against Gram-negative bacteremia. Increasing resistance to Fluoroquinolones is alarming. In future treating neutropenic patients with hematologic malignancies may deal with monitoring microbial gut diversity through treatment. Whether monitoring these microbial shifts help us treat or prevent MDR E. coli bacteremia in these patients remains unanswered.
Analysis of quinolone resistance due to mutational changes in escherichia coli associated with urinary tract infections: A study from North East India

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Background: Background: Quinolone group of antibiotics are predominantly used drugs for treatment of urinary tract infections. However, in recent decade's treatment failure to this drug is due to bacterial adaptation by acquisition of plasmid mediated quinolone resistance determinants. There also exists an intrinsic resistance mechanism by mutational changes in bacteria in type II and type IV bacterial topoisomerase. The study was designed to analysis point mutation in the gyrA and parC gene leading quinolone resistance in uropathogenic Esherichia coli from community settings.

Methods & Materials: Materials and methods: A total of 113 quinolone resistant enterobacterial isolates were collected from the urine of patient diagnosed with urinary tract infection from different community health centre of southern Assam during July 2013 to June 2014. All the isolates were investigated for mutations in the topoisomerases genes gyrA and parC by PCR amplification and amplicons were analysed for polymorphism by Denaturing gradient gel electrophoresis. MIC to fluoroquinolones was tested by the agar dilution method and results were interpreted as per CLSI guidelines.

Results: Results: Amongst the 113 enterobacterial isolates, 98 (86.72%) were resistant to norfloxacin, 92 (81.41%), 57 (50.44%) and 35(30.93%) showed reduced susceptibility to ciprofloxacin, ofloxacin and levofloxacin respectively. Several alterations were detected in gyrA and parC genes. Five different type of mutation in gyrA were detected in 94 isolates and 67 isolates were found to have mutation in parC. This study also demonstrated the mutation in quinolone resistance determining region of gyrA and/ or parC was associated with the MIC above the breakpoint for quinolone group of antibiotics.

Conclusion: Conclusion: The present study is of epidemiological interest and describes bacterial adaptation and genetic modification towards quinolone resistance. This study advocates urgent measures to be taken to stop over the counters availability of quinolone in this region and their misuse.
Microbiological profile of aerobic bacterial isolates causing complicated intra-abdominal infections managed at a tertiary level health care providing facility in Northern India

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**Background:** Complicated intra-abdominal infections (cIAIs) have been described by the surgical society (SIS), and the Infectious Disease Society of America (IDSA), as the infection of an abdominal organ that has spread to other abdominal structures. These infections range from uncomplicated appendicitis to fecal peritonitis. The leading site of pathology in our country, in contrast to western countries is gastro-duodenal perforations and enteric perforations whereas it is colonic perforation in western countries.

**Methods & Materials:** The present study was planned to determine the susceptibility profile of the various isolates from patients with cIAIs getting managed at Pt. B.D. Sharma, Post Graduate Institute of Medical Sciences, Rohtak, a tertiary level health care providing facility in northern region of India. The patients were selected based on pre-determined inclusion and exclusion criterion. Detailed clinical work-up and investigations were done at the time of admission. The peritoneal fluid/pus was sent for culture and sensitivity at the time of the intervention. The isolates were later identified following standard protocol and their antimicrobial susceptibility was determined by Kirby Bauer disk diffusion method, and the treatment where required was changed accordingly.

**Results:** A total of 114 intra-peritoneal fluid samples were submitted for microbiological evaluation. Out of these, 60 (52.6%) samples were sterile. A total of 57 aerobic bacterial isolates were recovered from 54 peritoneal fluid/pus samples. Escherichia coli (64.8%) was the commonest isolate recovered followed, by Klebsiella spp. in 12.2% of cases among the gram negative isolates. Enterococcus spp. was the commonest (41%) Gram positive isolate. Cefixime (93%) was the most effective antimicrobial agents in Escherichia coli followed by cefepime (88%) and co-amoxiclav (86%). Co-amoxiclav was the most effective drug in case of Klebsiella isolates (95%), followed by cefixime and ciprofloxacin (81%).

**Conclusion:** The antimicrobial susceptibility profile of bacterial isolates causing such infections can influence the final outcome in these conditions. Knowledge of the antimicrobial susceptibility profiles of the prevailing isolates can help in designing the empiric therapy for the cIAIs, which can influence the outcome of these infections favorably.
Antibiotics susceptibility pattern of Vibrio cholerae O1 Ogawa, isolated during Cholera outbreak investigations in Mozambique from 2014 to 2015
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Background:
Mozambique have registered cyclically epidemic outbreaks of cholera. Antibiotic therapy is recommended in specific situations for management and control of cholera outbreaks. However, an increase in antibiotics resistance rates of *Vibrio cholerae* has been reported in several epidemic outbreaks worldwide. On the other hand, there are few recent records of continuous surveillance of antibiotics susceptibility pattern of *Vibrio cholerae* in Mozambique.

Methods & Materials:
For susceptibility testing, we used *Vibrio cholerae O1 Ogawa* confirmed by culture, oxidase reaction and serology.² Samples were collected in the context of surveillance and response to Cholera outbreaks, in the period of January –April from the years 2014 and 2015; Sent to and tested in the National Reference Laboratory of Microbiology from the National Institute of Health – Mozambique.² Samples were from cholera treatment centers of Metangula (09), Mamba (01), Moatize (01), Morrumbala (01) districts, Tete City (08), City of Quelimane (01), Lichinga (06) and Nampula (86). Antibiotics susceptibility pattern was determined by disk diffusion method Kirby Bauer. Antibiotic susceptibility results were interpreted by following recommendations of CLSI (Clinical and Laboratory Standards Institute) 2014.

Results:
Among 117 isolates from *Vibrio cholerae O1 Ogawa* we found resistance of 100% (53/53) to trimethoprim-sulfamethoxazole, 100% (54/54) ampicillin, 99% (73/74) for Nalidixic Acid, 97% (64/66) to Chloramphenicol, 95% (42/44) for Nitrofurantoin, 82% (80/97) Tetracycline, 56% (39/70) Azithromycin and 0% (0/101) for Ciprofloxacin.

Conclusion:
Our work shows that the strains of the *Vibrio cholerae* isolated during outbreak investigation in Mozambique in the years 2014-2015 have a high frequency of resistance to available antibiotics, with the exception of Ciprofloxacin; and a rapid evolution of the resistance of antibiotics such as Nalidixic Acid, Chloramphenicol, Nitrofurantoin, and Tetracyclin. It's outstanding the appearance of resistant strains to Azithromycin, which is a recommended antibiotic for Cholera treatment. Since the appearance of this resistance can influence Cholera control strategies, continuous monitoring of epidemic strains is crucial.
Evidence of carriage of minimal form of resistance island in clinical isolates of multidrug-resistant Acinetobacter baumannii

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Background: Recent studies have recognized ATPase-encoding *comM* gene as a hot spot for integration of *Acinetobacter baumannii* resistance islands. Despite the circulating of high number of multidrug-resistant *A. baumannii* (MDR-AB) isolates in Middle East countries, no information is available about the carriage of resistance island.

Methods & Materials: The clonal type of 401 nonreplicate AB isolates was determined and the interruptions in *comM* gene as well as transposition were assessed.

Results: The most of MDR-AB isolates (384 of 388; 98%) and more than half of susceptible AB isolates (9 of 13; 69%) had interrupted *comM* gene carrying integrative element. All but 6 of global clone I (GC I) isolates (196, 97%) and 2 of GC II isolates (138, 98%) contained interrupted *comM* gene.

Conclusion: This study showed the carriage of interrupted *comM* in large series of isolates, indicating the presence of minimal form of resistance island.
Antibiotic susceptibility patterns of prevalent anaerobic gram negative bacilli in Lagos, Nigeria: A 20 year survey
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Background: The clinical significance of anaerobic bacteria is growing with advances in diagnostic technologies. Moreover, new treatment regimens are being introduced for the treatment of infections for which anaerobes are involved. It was therefore important to evaluate the impact of the changing patterns on antibiotic resistance in the Gram negative anaerobic bacteria. This report represents our experience with anaerobic infections in Nigeria from 1992-2011.

Methods & Materials: Data on prevalence and antibiotics susceptibility patterns of anaerobic bacteria in four specialist hospitals in Lagos, Nigeria from seven clinical conditions for which anaerobes were frequently isolated were analyzed. These include peritonitis following lower abdominal surgery (PLAS, 75 cases), periodontal abscess (PAB, 60), pelvic inflammatory disease (PID, 22), chronic suppurative otitis media (CSOM, 21), septic abortion (SAB, 16), dentoalveolar abscess (DAAB, 12), and bloodstream infections (BSI, 10). Data were analyzed in sub-sets of five years intervals; 1992-1996 (PA), 1997-2001 (PB), 2002-2006 (PC), and 2007-2011 (PD).

Results: The occurrences and distribution of the Gram negative bacilli (GNB) were Bacteroides, 251 (PA 45, PB 56, PC 67, PD 83), Fusobacterium, 151 (PA 34, PB 46, PC 56, PD 15), Porphyromonas, 30 (PA 3, PB 9, PC 17, PD 1), Prevotella, 127 (PA 36, PB 49, PC 34, PD 8). Fusobacterium and Porphyromonas were the most sensitive to antibiotics with no evident shift in pattern from 1992 to 2011 but showed highest sensitivity to the cephalosporins and metronidazole. Against Bacteroides and Prevotella, amoxicillin activity was least with no change in pattern over the study period. Slight but progressive resistance to the cephalosporins by Bacteroides and Prevotella occurred (22.7% for B. fragilis to ceftazidime in 1992-1996 to 28.6 % in 2007-2011). Metronidazole was the most effective antibiotics with resistance not higher than 22.7 % at any time by the most resistant species. The activities of the macrolides increased appreciably from 1992 to 2011 while amoxicillin-clavulanic acid activity was relatively constant.

Conclusion: These observations indicate that the changing pattern of antibiotic usage has not appreciably altered the antibiotic profile of anaerobic GNB in Nigeria.
Transcriptional response of arnA and pmrB in relation to polymyxin resistance in Pseudomonas aeruginosa associated with surgical wound infection: A study from North-East India

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Background: Introduction: Polymyxins were considered as the most effective drug against infections caused by multidrug resistant Pseudomonas aeruginosa. The increasing resistance mechanism by these organisms has led to the development of resistance towards this group of antibiotics. This study was designed to determine the prevalence of polymyxin resistant P. aeruginosa from North-East India and to detect the role of pmrB and arnA genes in resistant phenotype.

Methods & Materials: Methods and Materials: Consecutive non-duplicate clinical isolates of Pseudomonas aeruginosa were collected from the patients admitted to surgical ward of Silchar Medical College and Hospital in the duration of September 2013 to August 2014. The isolates were screened for polymyxin resistance by Kirby-Bauer disc diffusion method and the minimum inhibitory concentrations. mRNA and cDNA of five selected polymyxin resistant strains representing different MIC range were isolated in normal condition of the strain as well as after treating with FeCl₃ alone and FeCl₃ and polymyxin antibiotic. Transcriptional expression was observed for pmrB and arnA by quantitative real time PCR in reference to P. aeruginosa PAO1. Susceptibility pattern of these polymyxin resistant strains was performed by Kirby-Bauer disc diffusion method. DNA fingerprinting of the isolates was carried out by performing REP PCR.

Results: Results: A down regulated expression of pmrB and arnA was observed in polymyxin resistant strains of P. aeruginosa which is unique comparing to other studies. Low susceptibility rate to amikacin and gentamicin, β-lactam-β-lactamase inhibitor piperacillin-tazobactam and quinolone group of drug ciprofloxacin was shown by polymyxin resistant P. aeruginosa strains whereas total resistance was observed in case of third generation cephalosporin cefepime. REP PCR results showed these polymyxin resistant organisms are heterogenous by showing different clonal pattern.

Conclusion: Conclusion: This study highlights the urgency of obtaining knowledge on the pharmacology of polymyxins to optimize their clinical use and minimize potential for development of resistance. This will help in management of treatment and infection control due to multidrug resistant P. aeruginosa.
Molecular epidemiology and spread dynamics of multi-drug resistant in A. baumannii isolated from patients and hospital environment in Bangladesh

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**Background:** Multidrug-resistant (MDR) A. baumannii has been a serious challenge in the hospitals globally including Bangladesh. The study was performed to investigate molecular epidemiology & spread dynamics of antibiotic resistant A. baumannii both from patients and hospital environment in Bangladesh.

**Methods & Materials:** A set of 49 clinical *Acinetobacter* strains were collected from five clinical microbiology labs located in Dhaka, Bangladesh during 2014-2015. Additionally, 100 samples were collected from different hospital surfaces of Dhaka Medical College Hospital. All strains and samples were cultured on CHROMagar™ *Acinetobacter* selective media. *A. baumannii* was identified at species level by biochemical tests & `blaOXA-51` PCR. Resistance to 13 antibiotics under different 8 classes was determined according to EUCAST and CLSI. PCR was used to detect different resistance genes; quinolones (`qnrS1, aac6, qepA, qnrC1, qnrB1, qnrA1`), 16S methylase (`rmtA, rmtB, rmtC, rmtD, armA`)and OXAs(-23,-24,-58,-143). Real-time multiplex PCR were conducted for the presence of carbapenem resistant genes (`NDM, VIM, IMP, KPC` and `oxa-48`). Epidemiological typing & clonal profile was performed by rep-PCR.

**Results:** 95% (47/49) human and 31% (10/32) environmental isolates of *A. baumannii* had growth on CHROMagar™ agar. All clinical and 10 environmental strains carried `blaOXA-51` gene which confirmed as *A. baumannii*. Resistance to 4 or more antibiotic classes was found in 48 clinical and 10 environmental strains. Forty clinical and all environmental strains carried `blaOXA-23` however; `blaOXA-58` was in one clinical strain. The predominant ciprofloxacin resistant gene was `aac6` in both clinical and environmental isolates followed by `qnrB1` in clinical isolates and `qnrC1` in environmental isolate. Only `armA` gene was found in clinical and environmental strains. None of the clinical and environmental strains were positive for other carbapenem resistant genes (`NDM, VIM, IMP, KPC, OXA-48`). In total, 36 different clones were identified from both patients and environment; 6 different clinical clones (AC, BC, DC, FC, HC, PC) were common in different hospitals among patients. Some of the clones (CC, RC, P3, P6) were common both in patients and environmental strains.

**Conclusion:** The magnitude of resistance including their phenotypes and genotypes, and clonal relatedness among clinical and environmental *A. baumannii* indicates multi-drug resistant strains were wide spread in Bangladeshi hospitals.
Genotyping of mycobacterium tuberculosis strains isolated from patients with pulmonary drug resistant tuberculosis in Ukraine
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**Background:** The epidemiological situation of tuberculosis in Ukraine is extremely unfavorable – about 40 thousand people become sick on tuberculosis each year and about 10 thousand patients die. Changing of the contemporary socio-economic and environmental conditions plays an important role in the deterioration of the tuberculosis epidemic situation. Moreover drug resistance of *M. tuberculosis* is one of the main factors limiting the effectiveness of TB treatment. Due to the development of molecular genetics it has become possible to conduct genetic typing of bacteria, which allows distinguishing between different strains of the pathogen isolated from TB patients.

**Methods & Materials:** During 2014 the 93 cases of TB were studied in patients who were treated in hospitals in Kharkiv region in Ukraine. Mycobacterium identification and testing of the drug susceptibility were performed as recommended by WHO. The samples of expectoration were used for the strain isolation on Lowenstein-Jensen medium. VNTR genotyping was done by using sets of primers for amplification of five exact tandem repeat ETR loci as previously described (Frothingham, R., 1998).

**Results:** It has been shown that 69 % from isolated *M. tuberculosis* strains belong to Beijing family whereas 13% –to LAM. Other genotypes consisted 18% from all obtained isolates. The most common profile was 42435. The most common resistance among Beijing strains were observed to streptomycin (100%), followed by resistance to isoniazid (99%), rifampicin (96%) and ethambutol (89%). Kanamycin and ofloxacin did not inhibit growth of *M. tuberculosis* isolates in 69 % and 60 % cases respectively. The most effective anti-TB drug was cycloserine (10%). Among LAM strains it was found resistance to kanamycin, isoniazid and streptomycin in 12 (100%) cases, ethambutol and rifampicin – in 11 (91.5%) cases. The frequency of resistance to kanamycin, 4-Aminosalicylic acid and ethionamide was revealed significantly higher for LAM strain in compare to Beijing strain (p<0.05). 41 (44%) of 93 patients were diagnosed as MDR TB and 52 (56%) – TB with extending resistance.

**Conclusion:** In conclusion, the data showed that TB in Kharkiv region is mostly characterized as multi-drug resistance cases. To prevent circulation and transmission of *M.tuberculosis* TB control program needs to be improved in Ukraine.
Of bugs and drugs: have carbapenems met their doom?

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Background: Microorganisms though diminutive are formidable foes. The antibiotic soup that now permeates health-care facilities has exerted a selection pressure on pathogens and commensal organisms alike, and resistance has proliferated and spread such that many bacteria can withstand almost all drugs. Detection of β-lactamases by virtue of their diverse complexity is a diagnostic dilemma hindered by the heterogeneity of both host and enzyme. Expression of multiple β-lactamases and their co-existence is a common parlance particularly in nosocomial pathogens which are notorious for disseminating them compromising the therapeutic alternatives. When faced with this mélange of enzymes in resource constraint settings lack or inadequacy of diagnostic tests may have grave implications.

Methods & Materials: 168 clinical isolates E.coli (n=53) K.pneumoniae (n=115) from invasive site infections were studied for their antimicrobial susceptibility and detection of β-lactamase by inhibitor based disc potentiation test (DPT). A total of 18 agents of which 6 were substrate inhibitor combination discs were tested per isolate. Detection of ESBL, MBL, AmpC and KPC was done by using inhibitors- EDTA, Dipicolinic acid (DCA), Phenyl boronic acid (PBA), Clavulanic acid (CA) and cloxacillin(CLOX). Appropriate controls were incorporated.

Results: High resistance to all classes of cephalosporins was observed in all isolates. Carbapenem resistance was found in 87.15% isolates. Least resistance to cotrimoxazole and gentamicin was seen among the antimicrobials tested. ESBL was the most frequent β-lactamase detected followed by MBL>AmpC>KPC.

Conclusion: We are living in a world where we will never be able to stay ahead of the bacterial mutation curve. A test of our resilience is how far behind the curve we allow ourselves to fall. Limited data is available on presence of KPC’s in India and none from our region. There is a need for enhanced adherence with effective infection control measures, detect and protect strategy and antibiotic stewardship to curb patient-to-patient transmission and to reduce the selection of multidrug-resistant bacteria. Currently the main issues are providing practical recommendations on detection, treatment and prevention in different resources settings.


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Background: Streptococcus pneumoniae infections are challenging since pneumococci have more than 90 serotypes and emergence of resistant strains has increased. Surveillance systems are essential for effective vaccine strategies and development of treatment protocols. This study aimed to evaluate the serotype distribution and antimicrobial susceptibility of S. pneumoniae causing invasive pneumococcal disease in adults (≥18 years).

Methods & Materials: S. pneumoniae strains were collected from 14 different centers between 2005 and 2015. Pneumococcal serogroup and serotype identification was performed using standard conventional methods. Antibiotic susceptibility testing was performed using E-test and interpreted according to the CLSI 2014 standards.

Results: S. pneumonia strains (n=346) were isolated from blood (60.1%), bronchoalveolar lavage (19.7%), cerebrospinal fluid (11.0%), pleural fluid (6.1%), and other body fluids (3.1%). The most common S. pneumoniae serotypes were found as serotype 3 (13.0%), 19F (12.7%), 19A (6.1%), 14 (4.6%), and 6B (4.0%). Vaccine coverage rate was 27.4% for 7-valent pneumococcal conjugate vaccine (PCV-7), 53.5% for PCV-13, and 62.3% for 23-valent pneumococcal polysaccharide vaccine (PPV-23). For oral penicillin V, resistance rate was 21.7% and intermediate resistance rate was 16.8%. For parenteral penicillin, 52.6% was resistant in strains isolated from CSF (meningitis) and 0.6% was resistant and 5.8% was intermediate in strains isolated from other specimens. For cefotaxime, 5.3% was resistant and 18.4% was intermediate in strains isolated from CSF, whereas 1.6% was resistant and 6.5% was intermediate in strains isolated from other specimens. Erythromycin resistance was 28.6%. No resistance was detected to moxifloxacin but intermediate resistance was 0.6%. Serotypes 19A and 19F exhibited higher rates of penicillin and erythromycin resistances. Vaccine coverage rates for non-susceptible (resistant and intermediate) strains are presented in Table 1.

<table>
<thead>
<tr>
<th>Coverage rates for</th>
<th>Penicillin V (oral)</th>
<th>Penicillin parenteral (non-meningitis)</th>
<th>Penicillin parenteral (meningitis)</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV-7</td>
<td>52.0%</td>
<td>60.0%</td>
<td>40.0%</td>
<td>55.6%</td>
</tr>
<tr>
<td>PCV-13</td>
<td>68.6%</td>
<td>90.0%</td>
<td>65.0%</td>
<td>76.7%</td>
</tr>
<tr>
<td>PPV-23</td>
<td>74.7%</td>
<td>85.0%</td>
<td>75.0%</td>
<td>79.7%</td>
</tr>
</tbody>
</table>

Conclusion: Since serotype distribution and antimicrobial susceptibility of clinical S. pneumoniae isolates may change in time naturally also with medical interventions like antibiotic use and vaccination, close monitoring is essential.
Antibiotic susceptibility pattern of Brucella melitensis clinical isolates in Hamedan, West of Iran

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Background: Brucellosis is a widespread zoonotic disease with significant economic and major public health problem. Failure of response and relapse of brucellosis have reported with current therapeutic regimens. Considering the high prevalence of brucellosis and recurrence rate of this disease in Iran, widespread and inappropriate use of antibiotics could redound antibiotic resistance among Brucella isolates in the community. The aim of this study was to evaluate the antibiotic susceptibility pattern of Brucella isolates by E-test method in Hamadan, west of Iran.

Methods & Materials: In this study, patients with clinical diagnosis of brucellosis who referred to Infectious Diseases Ward, Sina hospital in Hamadan were studied between April 2013 and July 2014. Blood Specimens were collected for diagnosis of brucellosis by BACTEC blood culture system. Antimicrobial susceptibility patterns of clinical isolates to gentamicin, streptomycin, rifampin, doxycycline, ciprofloxacin, ofloxacin and trimethoprim-sulfamethoxazole were performed by the E-test method. Then the CLSI zone size and Minimum Inhibitory Concentration interpretive criteria were examined.

Results: One hundred forty-nine patients with brucellosis were enrolled in this study. Culture of clinical samples were positive in 38.3%, of which, 91.2% were associated with positive serological test. No significant associations were found between serological tests and culture method. All clinical isolates were sensitive to doxycycline, streptomycin, gentamicin, ciprofloxacin and moxifloxacin, but intermediate sensitivity to rifampin and trimethoprim-sulfamethoxazole were found in 35.08% and 3.5% of isolates, respectively.

Conclusion: Because of the high frequency of intermediate sensitivity to rifampin among brucella isolates, this drug should be prescribed with caution. We recommend restricting the use of rifampin for treatment of brucellosis except as an alternative drug for special situations.
In-vitro activity of Fosfomycin against clinical isolates of carbapenem resistant Acinetobacter baumannii complex and Pseudomonas aeruginosa at a South African academic hospital

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**Background:** Carbapenem resistant *Acinetobacter baumannii* complex and *Pseudomonas aeruginosa* are a major health problem in our hospital, most of them being multidrug resistant strains which are difficult to treat with the current available antimicrobial agents. This study was undertaken to evaluate the in-vitro activity of fosfomycin as alternative treatment against carbapenem resistant isolates.

**Methods & Materials:** Consecutive clinical isolates of carbapenem resistant *Acinetobacter baumannii* complex and *Pseudomonas aeruginosa* were collected over a 6 month study period. Species identification and antimicrobial susceptibility testing was performed using Vitek-2 colorimetric compact systems (BIOMERIUX, France). Fosfomycin testing was initially done by disk diffusion, thereafter the gradient diffusion method using the Etest strips (LIOFILCHEM, Italy) was done for the verification of the minimum inhibitory concentrations. The modified Hodge test was done to detect the presence of carbapenemases.

**Results:** A total of 133 carbapenems resistant clinical isolates (72 (54.1%) *Acinetobacter baumannii* and 61 (45.9) *Pseudomonas aeruginosa*), were collected from 125 patients. The majority of patients were males between 30-60yrs and 72 (57.6%) were in the ICUs, while 53 (42.4%) were in other wards. The specimens were from Endo-Tracheal Aspirates (ETA) 80 (60.1 %), 17 (12.8%) from blood culture, 16 (12%) from wound swabs, tissues and effusions, 11 (8.3%) from CVP tips and 9 (6.8%) from urine. All isolates were susceptible to fosfomycin in addition to colistin.

<table>
<thead>
<tr>
<th>ANTIBIOTIC</th>
<th>MIC range ug/ml</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colistin</td>
<td>≤ 2 - ≥ 4</td>
<td>100 %</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fosfomycin gradient diffusion</td>
<td>≤ 64 - ≥ 256</td>
<td>100 %</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fosfomycin disk diffusion</td>
<td>≤ 12 - ≥ 16</td>
<td>44.5%</td>
<td>47.2%</td>
<td>8.3%</td>
</tr>
</tbody>
</table>

**Conclusion:** In conclusion, treatment options for infections due to carbapenem and multi-drugs resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* organisms are limited, therefore fosfomycin may be considered as a therapeutic option for these organisms.
pncA mutations in mycobacterium tuberculosis is a strong predictor of poor treatment outcome in the therapy of multidrug resistant tuberculosis
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Background: Despite the widely accepted association between Pyrazinamide (PZA) resistance and mutations in pncA and rpsA gene, it remained unknown about the clinical value of PZA genotypic drug susceptibility testing (DST) for multidrug resistant tuberculosis (MDR-TB) treatment, especially in the resource-limited area, where the PZA phenotypic DST is difficult to be implemented. Thus this study was aimed at evaluating the role of PZA genotypic DST in predicting poor treatment outcome in a MDR-TB treatment cohort.

Methods & Materials: This is a cohort study of 74 MDR-TB patients who were identified and admitted to two Chinese designated hospitals in 2010-2011. Demographic and clinical information including treatment outcomes (sputum smear conversion and treatment success) were retrieved from medical records. Susceptibility to PZA and other 2nd line drugs were tested with the MGIT 960 PZA kit. Mutations in pncA and rpsA genes were identified by DNA sequencing.

Results: Among 74 MDR-TB cases, 27 (36.5%) were pre-XDR TB and 5 (6.8%) were XDR-TB. 44.6% (33/74) had PZA resistance with 84.8% (28/33) mutations in pncA gene and 3.0% (3.0%) mutation in rpsA gene. When comparing the treatment success reflected by smear negativity after two months of standardized MDR-TB therapy and a successful final outcome it is clearly seen that a pncA mutation predicted a less favorable response to treatment. A total of 21 (75%) of the patients having a strain with such a mutation were still smear positive after two months compared to 24 (53.3%) in the group with strains showing the pncA wt sequence (OR: 0.511; 95%CI: 0.262-1.084). Similarly results were seen for final treatment success (registered after 24 months), were 9/26 cases and 29/41 cases respectively responded successfully based on WHO guideline (OR: 0.107; 95%CI: 0.015-0.773).

Conclusion: This study suggest the sequencing of the pncA gene and its promoter can offer a rapid and reliable tool to predict a possible treatment failure in patients with MDR-TB.
Antimicrobial resistance of methicillin-resistant staphylococci isolated from food producing animal
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**Background:** The emergence of antibiotic-resistant microorganisms in food-animal environments poses a public health concern. Staphylococcal is an important opportunistic pathogen both in humans and in dairy cattle. It can be transferred between animals and humans either by direct contact or via the food chain. This study aimed to evaluate the phenotypic and genotypic characterization of methicillin-resistant staphylococci from dairy cattle in a rural community, Okada, Edo State, Nigeria.

**Methods & Materials:** A total of 283 samples from cattle (137 milk samples and 146 nasal swabs) were assessed between February and April 2015. Phenotypic reactions were conducted using Modified Hodge Test (MHT), and Double Disk Synergistic Test (DDST). Antimicrobial susceptibility was performed by Kirby-Bauer disk diffusion method according to CLSI standard. Polymerase chain reaction (PCR) assay were employed for the detection of 16S rRNA, **mec**A, **nuc** and PVL genes.

**Results:** The Staphylococcal was identified through partial 16S ribosomal ribonucleic acids (rRNA) nucleotide sequencing and BLAST analysis of the gene sequence showed the staphylococcal to have 98% - 100% similarity to *Staphylococcus aureus* (30), *S. epidermidis* (17), *S. haemolyticus* (15), *S. saprophyticus* (13), *S. chromogenes* (8), *S. simulans* (7), *S. intermedius* (6) and *S. xylosus* (4). MHT showed 90% and 96% of the isolates from nasal and raw milk respectively, revealed carbapenemase activity. Also, 4% of the isolates from the nasal cavity and 8% of the isolates from raw milk were positive to DDST, thus revealing metallo-β-lactamases production. Resistance of 100% was observed in all *Staphylococcus* spp., against MET, PEN, CLN, CHL and SXT. Multidrug resistant from nasal cavity and raw milk reveals 13 isolates against **MET**R, **PEN**R, **AMX**R, **CLN**R, **CHL**R, **SXT**R, **CLX**R, **KAN**R, **ERY**R, and **VAN**R. Of all isolates, 100% harboured the **mec**A, 46% of the isolates **nuc** genes while 30% of the isolates possess the PVL gene. All *S. aureus* isolates harboured the PVL gene while other *Staphylococcus* spp., were negative to the PVL gene. *S. aureus* were 90% positive to the **nuc** gene.

**Conclusion:** The presence of methicillin-resistant *Staphylococcus* isolates in dairy cattle is a potential public health risk and thus the findings in this study can be used as a baseline for further surveillance.
Antibiogram characterization of salmonella serovars isolated from food-animal and abattoir effluents
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Background: Salmonella species have been incriminated globally as a leading cause of gastroenteritis in humans, with food producing animals serving as potential reservoirs. The widespread of antimicrobial-resistant Salmonella has been a serious human and animal health problem. The objective of this study was to evaluate Salmonella serovars recovered from slaughtered food-animals and wastewater in abattoir environment.

Methods & Materials: This study was conducted between May and July 2015 in Benin City. A total of 170 samples, comprising of 25 each from rectum, ileum and gall bladder of cattle and 15 each from the same anatomical sites of goats and 50 environmental samples from abattoir effluents. The samples were collected from different abattoirs in Benin City environs. Samples were analyzed for Salmonella using the standard culture-based, biochemical and serological techniques. Salmonella colonies were screened using species-specific primers (Salmonella Enteritidis-ENT-F&ENT-R and Salmonella Typhimurium (STM4492-F&STM4492-R) employing PCR assay. Antibiotic susceptibility of the isolates was determined by the Kirby-Bauer disc-diffusion method following the CLSI guidelines.

Results: A total of 170 samples were analyzed, the overall prevalence rate of Salmonella species revealed 98(57.64%) in all samples analysed with Salmonella Enteritidis 63(37.05%) and Salmonella Typhimurium 69(40.58%). Salmonella Enteritidis and Salmonella Typhimurium from food-animals and abattoir effluents exhibited 100% resistance against chloramphenicol, ampicillin, erythromycin and tetracycline. High resistance was observed against amoxicillin/clavulanic acid (92.3%), streptomycin (87.3%) and sulfamethoxazole (85.2%) for both Salmonella serovar isolates. Salmonella serovar isolates were susceptible to amikacin (98%), ciprofloxacin (95.8%), gentamicin (89.5%) ofloxacin (89.3%) and cephalothin (85%). Multidrug resistance (to three or more antibiotics) was recorded in Salmonella Enteritidis 58.73% (37/63) and Salmonella Typhimurium 60.86% (42/69). Salmonella serovar isolates exhibited multiple antibiotic resistances (MAR) index between 0.250 and 0.583. Food-animals and abattoir effluents were observed to be potential reservoirs of Salmonella serovars.

Conclusion: This study has revealed the importance of monitoring antimicrobial resistance in Salmonella associated with food animals and the environment. It further implies that foods-animals could be vital source, if not properly monitored transmission of salmonellosis to humans.
AmpC β-lactamase producing Escherichia coli associated with urinary tract infection from a tertiary health care centre in North East India.

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Background: Escherichia coli are the major causes of urinary tract infection (UTI) and an emerging problem worldwide. These organisms harbouring AmpC beta-lactamase is a major cause of therapeutic failure leaving cephalosporins inactive along with co-existing mechanism of resistance. The aim of this study was to investigate the prevalence of Escherichia coli producing AmpC beta-lactamase in UTI and analyse their antibiotic susceptibility pattern from a tertiary care hospital in North Eastern part of India.

Methods & Materials: A total of 126 Escherichia coli were biochemically identified from a 293 consecutive non-repetitive clinical isolates which were obtained from patient with UTI over a period of 12 months (February 2013 to January 2014). Isolates were initially screened with cefoxitin (30 µg) and confirmed for production of AmpC beta-lactamases by M3DET. Phenotypically positive isolates were further confirmed by multiplex PCR targeting blaCIT, blaACC, blaEBC, blaDHA, blaFOX, blaMOX families. Antimicrobial susceptibility and MIC’s were also determined as per CLSI guideline.

Results: Out of 126 Escherichia coli isolates 97 (76.9%) were found to be cefoxitin resistant and among them 46 (47.4%) isolates were confirmed to be AmpC producers. Most of the AmpC positive isolates were found to be significantly multi-drug resistant and their minimum inhibition concentrations were above the break point. Out of 46 isolates 39 (84.7%) showed genotypically positive for blaAmpC genes (24CIT, 12DHA and 3EBC).

Conclusion: In the present study AmpC β-lactamase production is accompanied by multi drug resistance therefore, therapeutic options become limited resulting a need for new measures for the management of Escherichia coli causing urinary tract infections.
Antibiotic resistance among hospitalized patients in Mauritius in 2014

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Background: A retrospective survey of bacteria isolated from hospitalised patients in Mauritius in July 2014 was conducted to obtain updated information on the extent of antimicrobial resistance in the country.

Methods & Materials: Results of all specimens of urine, pus swab, blood culture, tracheal secretion and intravascular catheter originating from hospital wards and processed at the Central Health Laboratory and at Jeetoo Hospital Laboratory in July 2014 were reviewed and analysed. Duplicate isolates from the same patient were excluded. Organisms considered likely to be normal flora or contaminants were not considered.

Results: Of 686 gram-negative bacteria recorded, the most common were *E. coli* (183), *Klebsiella* spp. (118), *Pseudomonas aeruginosa* (93), *Acinetobacter* spp. (78) and *Proteus* spp. (61). Most isolates were obtained from pus swabs (47%) and urine (25%) but tracheal secretion was the source of 32% of *Acinetobacter* spp. Percentage susceptibility to ceftotaxime, ciprofloxacin, gentamicin, amikacin, meropenem and colistin was 54%, 42%, 73%, 93%, 97% and 100% respectively in *E. coli*, with corresponding figures of 42%, 47%, 57%, 81%, 91% and 100% respectively in *Klebsiella* spp., 6%, 18%, 21%, 42%, 26% and 100% respectively in *Acinetobacter* spp., and 64%, 72%, 64%, 89%, 100% and 0% respectively in *Proteus* spp. Less than three-quarters of *P. aeruginosa* isolates were susceptible to gentamicin (58%), ciprofloxacin (53%), ceftazidime (70%), amikacin (71%), imipenem (73%) and meropenem (73%) but 89% were susceptible to piperacillin-tazobactam combination and 100% to colistin. Of 308 significant Gram-positive isolates recorded, *Staphylococcus aureus* (140) and *Enterococcus* spp. (96) were most commonly encountered. Only 61% of *S. aureus* isolates were susceptible to methicillin, but 69% and 100% were susceptible to erythromycin and vancomycin respectively. *S. aureus* was isolated from blood cultures of 27 patients, of which 5 (19%) were methicillin-resistant (MRSA). Of 96 enterococci recorded, 92% were susceptible to ampicillin and none was vancomycin-resistant.

Conclusion: Resistance to broad-spectrum antibiotics is common among Gram-negative bacteria and *S. aureus* isolated from hospitalised patients in Mauritius. Therapeutic options to treat many infections in hospitalized patients are now limited. There is a need for effective infection control and antibiotic stewardship programs to slow the emergence and spread of microorganisms with acquired resistance mechanisms.
In vitro antimicrobial susceptibility of ceftriaxone/sulbactam/ethylenediaminetetraacetic acid and comparison to other beta-lactam/beta-lactamase inhibitors, carbapenems and colistin against gram negative bacteria

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Background: Drug resistance against Gram Negative Bacteria (GNB) is increasing. Incidence of ESBL producing bacteria is around 70-80%. Carbapenem resistance has also been reached 40-90% for the GNB. We are also obtaining Colistin resistant isolates. Resistance against Beta-lactam (BL)/beta-lactamase inhibitor (BLI) combinations is already very high. No new antibiotic or antibiotic group is in pipeline at least for the next 5-10 years. With this background the objective of this study is to compare in vitro susceptibility of new BL/BLI combination Ceftriaxone/Sulbactam/Ethylenediaminetetraacetic Acid (CSE) to Piperacillin/Tazobactam, Cefoperazone/Sulbactam, Cefepime/Tazobactam, Meropenem, Imipenem and Colistin.

Methods & Materials: Study was conducted on all clinical samples received from all critical care units between January 2014 and June 2015. Identification and susceptibility was done by Vitek 2 compact system. Susceptibilities of Ceftriaxone/Sulbactam/Ethylenediaminetetraacetic Acid and Cefepime/Tazobactam were done by disc diffusion method on the bases of CLSI guidelines. Escherichia coli, Klebsiella sp., Pseudomonas sp. and Acinetobacter sp. isolates were included in the study.

Results: Escherichia coli (324, 25%) was the most common bacteria isolated followed by Klebsiella sp. (309, 24%), Pseudomonas sp. (217, 17%) and Acinetobacter sp. (214, 17%) from all clinical samples. % Susceptibilities were as given in table below.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of isolates</th>
<th>CS E</th>
<th>Cefepime/Tazobactam</th>
<th>Piperacillin/Tazobactam</th>
<th>Cefoperazone/Sulbactam</th>
<th>Meropenem</th>
<th>Imipenem</th>
<th>Colistin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>324</td>
<td>67.4</td>
<td>77.3</td>
<td>46.5</td>
<td>57.9</td>
<td>73.1</td>
<td>72.7</td>
<td>99.5</td>
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<tr>
<td>Klebsiella sp.</td>
<td>309</td>
<td>28.3</td>
<td>37.6</td>
<td>18.6</td>
<td>26.2</td>
<td>32</td>
<td>32</td>
<td>70.9</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>217</td>
<td>43.1</td>
<td>64.1</td>
<td>40.6</td>
<td>46.9</td>
<td>52.4</td>
<td>50.3</td>
<td>93.8</td>
</tr>
<tr>
<td>Acinetobacter sp.</td>
<td>214</td>
<td>24.3</td>
<td>13.9</td>
<td>11.3</td>
<td>23.6</td>
<td>12.5</td>
<td>11.8</td>
<td>95.8</td>
</tr>
</tbody>
</table>

Conclusion: Colistin was the most sensitive antimicrobial for all GNB. Carbapenem resistance was around 27% - 89%. CSE susceptibility was better than Piperacillin/Tazobactam and Cefoperazone/Sulbactam and comparable to Meropenem and Imipenem. Although the number of isolates included in this study were less in number, a larger study needs to be conducted. This is an in vitro susceptibility data hence study has to be conducted for clinical efficiency of CSE.
Antimicrobial susceptibility and serotype distribution of invasive and non-invasive streptococcus pneumoniae isolates and comparison from healthy carriers

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Background: Streptococcus pneumoniae is a major cause of morbidity and mortality in developing countries. S. pneumoniae as a commensal in nasopharynx plays a significant role in infection. This study was undertaken to determine the antimicrobial susceptibility pattern and serotype distribution of S. pneumoniae isolates from invasive & non invasive infection and correlate it with isolates from commensal nasopharyngeal flora to ascertain their role in infection in our population.

Methods & Materials: S. pneumoniae isolates from Blood/CSF and respiratory secretions (sputum, BAL and nasopharyngeal swab/throat swab) were analyzed to determine serotype and antimicrobial susceptibility pattern. Serotyping was performed by the quellung reaction. Antimicrobial susceptibility testing was determined using disk diffusion method against the following antibiotics: Penicillin (1µg oxacillin), Amoxicillin (1µg), Amoxiclav (30µg), Vancomycin (30µg), Erythromycin (15µg), Tetracycline (30µg), Levofloxacin (5µg), Ofloxacin (5µg), Gatifloxacin (5µg), Trimethoprim-sulfamethoxazole (1.25/23.75µg), Chloramphenicol (30µg), Linezolid (30µg) as per CLSI guidelines. Minimum inhibitory concentration was determined using E-test for penicillin. S. pneumoniae ATCC 49619 was used as a control.

Results: A total of 31 isolates were collected including 11 from sputum, 15 from throat swab/nasopharyngeal swab, 1 from BAL and 4 from CSF. Invasive isolates belonged to serotypes 14, 3 & 15B. The most frequent serotypes among isolates obtained from sputum were 7, 22 & 35. Isolates from nasopharyngeal swab/throat swab belonged to serotypes 6, 19, 1 & 11 out of which serotype 6 was the most common. Two isolates were non-typable. Out of 31 pneumococcal isolates 3(9.6%), 7 (22.5%) and 28 (90.3%) were resistant to penicillin, erythromycin and trimethoprim-sulfamethoxazole respectively. Oxacillin resistant isolates were further tested for penicillin susceptibility by E-test. All 3 oxacillin resistant isolates demonstrated intermediate resistance to penicillin. These 3 isolates were non-invasive. All isolates were susceptible to vancomycin, linezolid & amoxicillin-clavulanic acid. It is difficult to isolate invasive S. pneumoniae from clinical samples therefore non-invasive isolates from sputum can be good surrogate for antibiotic resistance monitory.

Conclusion: In present study although the number of isolates are small, increase in the incidence of penicillin resistance is worrisome. There is a need to monitor penicillin resistance among invasive & non-invasive pneumococcal isolates. Our study implies the importance of continuous screening of serotypes & antimicrobial susceptibility pattern of S. pneumoniae isolates.
Healthy carriage of drug resistant Enterobacteriaceae in the community

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Background:
The emergence of resistance in the normal flora is a major concern in the management of both hospital and community acquired infections. The prevalence of the resistant organisms in the community is on the rise. The selection pressure is causing colonization resistance leading to blurring of distinction between community and hospital environments. Thus this study was conducted to find out the prevalence of resistant organisms in the normal flora of healthy population.

Methods & Materials: A community based surveillance was done from August to October, 2015. 207 stool samples were collected from the residents of Burail. The population under study was evenly distributed amongst paediatric and adult age groups. The samples were inoculated on antibiotic containing media which included cefotaxime, ceftazidime, cefepime, ciprofloxacin, imipenem, meropenem, amikacin and gentamicin. Molecular methods included multiplex PCR targeting TEM, OXA-1, CTXM-1, SHV and VIM genes.

Results: Out of the total number of 207 stool samples, E. coli was the predominant isolate followed by K. pneumoniae, Enterobacter species and Morganella morgannii. The resistance to cephalosporins was observed to be highest in E. coli (56%), followed by K. pneumoniae (0.04%). TEM was the predominant ESBL (34.9%) followed by OXA-1 (26%), CTXM1 (21.2%) and SHV (6.8%). Three samples grew CRE with VIM as the carbapenemase in one isolate only. Other antibiotics to which these isolates were resistant included fluoroquinolones (42%) wherein E. coli accounted for 40%; and aminoglycosides (0.01%). Eleven isolates (0.05%) were found to be multidrug resistant of which E. coli accounted for 0.04% and K. pneumoniae (0.01%).

Conclusion: The community based surveillance shows the presence of antimicrobial resistance being present in the normal colonic flora. Majority of the population under study was neither on prior antibiotic therapy nor hospitalized in the past 1 year. E. coli was the predominant isolate obtained with the maximum resistance to cephalosporins. ESBL was the commonest mechanism of resistance with TEM being the most prevalent
Epidemiology of extended-spectrum beta-lactamase- and carbapenemase-producing bacteria in stool from apparently healthy children, South Africa

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Background: We aimed to determine the prevalence and genetic characteristics of extended-spectrum beta-lactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae in stools from healthy infants and their mothers, and to determine the risk factors associated with their carriage.

Methods & Materials: This study was nested within the Drakenstein Child Health Study, South Africa. Maternal and infant faecal samples were collected at birth and at two additional time-points from the infants. Samples were screened for ESBLs and carbapenemase-producing organisms using ChromID ESBL and ChromID CARBA media, respectively. Vitek 2 was used for antibiotic susceptibility testing and identification of suspect ESBL/carbapenemase-producing isolates. ESBL production was confirmed using the combination disc test, and carbapenemase production using the modified Hodge test. Selected ESBL and carbapenemase genes were characterized by singleplex polymerase chain reaction and Sanger sequencing.

Results: Maternal faecal carriage of ESBL-producing bacteria was 4.4%; 4/90 (95% confidence interval (CI): 1.5% - 10.2%), and that of infants at birth was 3.5%; 4/116 (95% CI: 1.2% - 8.0%). The infant faecal carriage of ESBL-producing bacteria at 5-12 and 20-28 weeks was 4.4%; 3/68 (95% CI: 1.3% - 11.3%) and 5.7%; 2/35 (95% CI: 1.2% - 17.1%), respectively. ESBL genes were detected in six K. pneumoniae (blaCTX-M-15), five E. cloacae (blaSHV-12 and blaSHV-5) and three E. coli (blaCTX-M-14) isolates. No carbapenemase-producing bacteria were identified in this study. Pulsed-field gel electrophoresis showed heterogeneous clones of CTX-M-15-producing K. pneumoniae isolates. In contrast, in a mother-infant pair, we observed clonal relations among ESBL-producing E. cloacae isolates. In addition, one infant was persistently colonized by SHV-12-producing E. cloacae. Univariate analysis showed that infants born to HIV-positive mother, via elective caesarean section, and medication use before discharge were positively associated with ESBL faecal carriage. In contrast, breastfeeding prior to discharge was negatively associated with ESBL carriage.

Conclusion: This is the first study to detect ESBL-producing bacteria in human meconium samples in Africa, and raises questions on the source of such isolates and implications for community transmission. Further research is required to determine the spread of ESBL- and carbapenemase-producing bacteria in community settings in South Africa.
Characterization of carbapenem resistance in clinical isolates of Enterobacteriaceae

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Background: Emergence of carbapenem resistance among clinical isolates of Enterobacteriaceae pose a major public health concern globally. Early and accurate detection of patients harboring carbapenem non-susceptible bacteria is crucial in hospital infection control and resistance transmittance between patients. Understanding the common mechanisms of resistance determinants of these bacteria is pivotal in developing effective screening methods in the laboratory.

Methods & Materials: A laboratory based exploratory study was undertaken in a clinical microbiology laboratory attached to a tertiary care teaching hospital in south India. Clinical isolates belonging to family Enterobacteriaceae that showed resistance to meropenem by Kirby Bauer disk diffusion testing were included in the study. Modified Hodge test (MHT), Metallo beta lactamase –double disk synergy testing (MBL-DDST) and MBL-combined disk test (MBL- CDT) were employed for phenotypic detection of carbapenamase production. A multiplex PCR targeting the detection of blaNDM, blaKPC, blaVIM, blaIMP and blaGIM genes was used for genotypic confirmation of resistance and detection of the resistance determinants.

Results: A total of 64 non-repetitive Enterobacteriaceae isolates comprising of 45 (70.3%) Klebsiella pneumoniae, 12 (18.8%) Escherichia coli and 7 (10.9%) Enterobacter spp., were included in the study. NDM, KPC, VIM, IMP were the resistance determinants detected using PCR among 50 (78%), 39 (60.9%), 4 (6.3%) and 3 (4.7%) of the 64 isolates tested. Presence of both NDM and KPC was observed in 34 (53%) isolates. Further, 13 (20.3%) and 42 (66.7%) of the isolates were ESBL and AmpC producers. MHT, MBL-CDT and MBL-DDST could detect carbapenamase production in 62 (96.9%), 64 (100%) and 50 (79.4%) of the isolates tested. MBL-CD test could detect carbapenamase production due to both KPC and MBL class of enzymes in all isolates, while MHT failed in detecting carbapenamase production among 2 isolates that were NDM and KPC positive by PCR.

Conclusion: From the present study, we observed that NDM and KPC are the major resistance determinants for carbapenem non-susceptibility in our settings. Further, we noticed the supremacy of MBL-CDT over MHT and MBL-DDST screening methods for detection of carbapenamase production among Enterobacteriaceae isolates.
Multidrug resistant blood culture isolates: An experience from a tertiary care hospital in Eastern Nepal

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Background: Blood stream infections (BSI) remain a major cause of morbidity and mortality. Emergence of resistant microorganisms causing BSI has posed significant challenge to the clinicians and microbiologists alike. Surveillance of antimicrobial resistance is of utmost importance to track changes in microbial population, to select the appropriate therapy and to make recommendation in policy making. Present study was aimed to evaluate the antimicrobial resistance of blood culture isolates at BP Koirala Institute of Health Sciences (BPKIHS), a tertiary care teaching hospital in eastern Nepal.

Methods & Materials: Blood culture specimens submitted to Department of Microbiology were evaluated. Isolation, identification and determination of antimicrobial susceptibility was performed by standard microbiological techniques.

Results: Out of 11264 blood culture specimens processed over one year period, 1551 (13.8%) yielded the growth. Commonly isolated organisms in the descending order of frequency were Staphylococcus aureus (43%), Acinetobacter (19%), Enterococci (13%), Klebsiella (8%), Pseudomonas aeruginosa (7%), Escherichia coli (5%), Citrobacter (2%), Salmonella (1%), Proteus (1%) and Enterobacter (1%). Resistance to antimicrobials in common use was observed in varying frequency. MRSA was 40% whereas resistance to vancomycin and linezolid was not present. Resistance to ampicillin, ciprofloxacin and vancomycin was observed in 75%, 45% and 10% of Enterococcal strains respectively. 30% were HLGR whereas all remained susceptible to linezolid. Among the Enterobacteriaceae, production of ESBL, carbapenemase and AmpC were detected in 49%, 26.5% and 5% respectively. Of 51 carbapenemase producers, 11.5% produced MBL. K1 β-lactamase was not produced by any isolates of Enterobacteriaceae. Co-production of ESBL + carbapenemase was detected in 5% whereas ESBL + AmpC was seen in 0.5%. 33.4% of Enterobacteriaceae were MDR. Carbapenemase production was detected in 5% of Pseudomonas and 10% of Acinetobacter respectively whereas as 35% of both were ESBL producers. Overall 17% all gramnegative bacilli were tigecycline resistant.

Conclusion: Both gram positive and gram negative bacteria were responsible for bloodstream infections. Significant degree of antimicrobial resistance with emergence of multiresistance among these isolates over time is a matter of concern. Strengthening of ongoing antimicrobial surveillance system for early detection of resistant isolates is imperative for appropriate selection of antimicrobial therapy and prevention of spread of resistance.
Background: Numbers of patients with diabetic gangrene is increasing. Antimicrobial treatment is commonly used, however, limb amputation cannot be avoided in severe cases. For prophylaxis at operation, basic antimicrobial agents such as cefazolin are often administrated, however, severe infection could occur if resistant strains were cultured especially in immunosuppressive patients such as diabetes. The purpose of this study is to clarify bacterial species and their susceptibility to antimicrobial agents for patients of diabetic foot gangrene.

Methods & Materials: Twenty-four patients (nine females) who had amputation on their legs for treating diabetic gangrene were enrolled from year 2002 to 2012. Among them, fifteen patients had diabetic history for over ten years, eleven patients were having repetitive hemodialysis. Their ages were 40-81 (mean 67), average hospitalization period were 81.6 days.

Results: As results, fifty-seven strains were isolated. Among them, 62% strains were Gram-positive cocci, 33% were Gram-negative rods, 5% were Gram-positive rods. Two or more strains were detected in fifteen patients. Indigenous bacteria of skin such as MSSA were most commonly cultured (n=10). MRSA was found only in one patient. Sixty-seven percent of Peptostreptococci (n=7), and seventy-five percent of E.coli (n=4) were resistant to new quinolones. All Enterococci (n=6) were susceptible to penicillin. Seventy percent of operated patients had no complications and discharged normally. Others had re-operation, including two cases dead due to heart disease. Most commonly used antimicrobial agents for prophylaxis were cefazolin (n=8). However, sixty percent of all operated cases had resistant bacterial strain against cefazolin.

Conclusion: We conclude that in order to avoid inappropriate anti-microbial therapy, it is important to confirm antimicrobial susceptibility with bacterial culture before operation. Use of cefazolin as the first choice prophylaxis antimicrobials should be re-considered for diabetic gangrene amputation.
The species distribution and resistance pattern of vancomycin resistance enterococci from bloodstream infections in Istanbul, Turkey
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Background: Vancomycin-resistant enterococci, particularly Enterococcus faecium, are still one of the major causes of nosocomial infections worldwide, due to its typical multi-drug resistance profile and the tendency to create serious infections in critically ill patients. The aim of this study is to determine species distribution and resistance pattern of vancomycin resistance enterococci isolates from bloodstream infections at Istanbul University Cerrahpasa Medical Hospital.

Methods & Materials: Between January 2014 and October 2015, a total of 97 enterococci were isolated from blood samples of hospitalized patients with true bacteremia in intensive care units and in other departments of our hospital. Blood cultures were analyzed with the BACTEC 9120 system (Becton Dickinson, USA). The identification and antimicrobial resistance of isolates were determined by Phoenix automated system (BD Diagnostic Systems, Sparks, MD).

Results: The species distribution of enterococci was as follows: Enterococcus faecalis 53 (54.6%) and Enterococcus faecium 44 (45%). Resistance to vancomycin was detected in 16% of E. faecium isolates. None of E. faecalis isolates were resistant to vancomycin. Resistance rates of E. faecium and E. faecalis isolates to the antibacterial agents, respectively, were as follows: ampicillin 75% and 6%, high-level gentamicin 27% and 26%, high-level streptomycin 25% and 41.5%, linezolid 2% and 0%, norfloxacin 25% and 34%. None of the isolates were resistant to daptomycin and quinupristin/dalfopristin.

Conclusion: Our results showed that sixteen percent of our bloodstream E. faecium isolates were resistant to vancomycin and this situation highlights the importance of strict implementation of antibiotic policies to prevent emergence and spread of vancomycin resistant enterococci.
Detection of carbapenemase genes OXA-48, VIM, IMP, KPC and NDM in carbapenemase-producing klebsiella pneumoniae isolates from blood cultures of hospitalized patients in Istanbul, Turkey

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Background: Carbapenemase-producing Klebsiella pneumoniae (CPK) isolates have emerged as major causes of healthcare-associated infections worldwide. The present study was conducted to investigate the most prevalent carbapenemase genes; blaOXA-48, blaVIM, blalMP, blaNDM and blakPC in CPK isolates from blood culture of hospitalized patients at Istanbul University Cerrahpasa Medical School hospital.

Methods & Materials: Between January 2012 and October 2015, a total of 100 CPK isolates were isolated from blood culture samples of hospitalized patients with bacteremia in intensive care units and in various departments of our hospital. Blood cultures were analyzed with the BACTEC system (Becton Dickinson, USA). The identification and antimicrobial resistance of CPK isolates were determined by Phoenix automated system (BD Diagnostic Systems, Sparks, MD). The detection of carbapenemase genes was performed by real-time PCR using MDR KPC/OXA Real-TM and MDR MBL (VIM, IMP, NDM) Real-TM PCR kit (Sacace Biotechnologie, Italie).

Results: ESBL rate was 87% in CPK isolates. All isolates were phenotypically positive for carbapenemase activity. The isolates were highly resistant to cefuroxime (96%), and amoxicillin/clavulanic acid (91%), ceftriaxone, cefotaxime, ceftazidime (87%), piperacillin/tazobactam (86%), cefepime (82%), ciprofloxacin (76%) and gentamicin (65%). Amikacin resistance rate was 18%. Colistin and tigecycline resistance rates were 1%. The carbapenemase gene blaoxa-48 was detected in 43% of isolates and blavim in 5%. One isolate harbored a combination of blaoxa-48 and blavim. None of the isolates harbored blandm, blakpc or blalmp.

Conclusion: The carbapenemase genes blaoxa-48 with blavim was isolated in one of our isolates. Significant effort must be made to prevent the spread of CPK and continuous monitoring of drug resistance is necessary in our clinical settings.
Detection of IMP, VIM and NDM metallo-beta-lactamase carbapenemase genes in carbapenem resistant pseudomonas strains from bloodstream infections in Istanbul, Turkey
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Background: Carbapenem-resistant Pseudomonas aeruginosa causing various life-threatening infections is an important problem worldwide. Carbapenemase genes are one of the most common mechanisms reported in carbapenem-resistant P. aeruginosa. In this study, we aimed to determine the presence of IMP, VIM and NDM metallo-beta-lactamase (MBL) carbapenemase genes.

Methods & Materials: Between 2011 and 2015, a total of 48 carbapenem-resistant/intermediate Pseudomonas strains were isolated from blood samples of patients with bacteremia who were hospitalized in intensive care units and in various departments at Istanbul University Cerrahpasa Medical Faculty hospital. Blood cultures were analyzed with the BACTEC system (Becton Dickinson, USA). The identification and antimicrobial resistance of the strains were determined by Phoenix automated system (BD Diagnostic Systems, Sparks, MD). Phenotypic MBL E-test was used to investigate the presence of MBL enzymes. All study strains were screened by multiplex PCR for the presence of MBL genes.

Results: The species distribution was as follows: P. aeruginosa 42 (87.5%), P. putida 5 (10.4%) and P. fluorescens 1 (2.1%). Thirty six (76%) of isolates were carbapenem-resistant (MIC ≥32 µg/mL), 12 (25%) intermediate resistant (MIC 6–31 µg/mL). MIC50 and MIC90 were respectively 32 µg/mL and 32 µg/mL to both imipenem and meropenem. Resistance rates of the isolates to the antibacterial agents, respectively, were as follows: sefepim and ticarcillin 56%, levofloxacin 52%, ciprofloxacin 50%, ceftazidime 48%, piperacillin/tazobactam 46%, tobramycin and netilmycin 44%, gentamicin and aztreonam 37.5%, and amikacin 29%. None of the isolates were resistant to colistin. MBL screening with EDTA was positive in 6.3% (n = 3). In these three isolates with MBL positive, VIM type MBL gene was detected in two P. aeruginosa and one P. putida. NDM and IPM-type MBL genes were not in any of the isolates.

Conclusion: In our study, VIM type MBL gene was first shown in Pseudomonas strains at our hospital. Therefore, identification of carbapenemase genes restricting treatment options is important to rational antibiotic use and also to implement infection prevention measures to reduce their spreading.
Carbapenemase producing enterobacteriaceae from chronic hemo-dialysis and renal transplant patients from a tertiary care centre in Chennai, South India
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Background: Carbapenemase producing Enterobacteriaceae (CPE) is on the rise worldwide. They are currently reported from all the continents in a variety of environmental settings. Different carbapenemases have geographical preponderance with the blaNDM and blaOXA48 like being prevalent in the Indian sub continent. Although many Indian studies have reported their prevalence in the hospital settings, systematic studies on their prevalence in the community are lacking. Chronic Hemo Dialysis (CHD) patients and renal transplant (RT) patients are a unique group with constant interaction with both the community and hospital settings. Hence, the current study was designed to find the prevalence of CPE and their associated resistant genes from Chronic Hemo Dialysis and renal transplant patients from a tertiary care centre in Chennai, South India.

Methods & Materials: A total of 315 non-repetitive enterobacterial isolates from CHD and RT patients were included in this study. They were screened for carbapenem resistance and carbapenemase production by antibiotic sensitivity testing and Modified Hodge Test respectively. The isolates were identified by conventional biochemical methods. The carbapenem resistant isolates were screened for the presence of genes encoding carbapenemases, Extended Spectrum Beta Lactamases, plasmid AMPCs, 16S rRNA methylases and PMQR genes by PCR using published methods.

Results: 32/315 (10%) isolates were found to be CPE isolates of which 23 were K.pneumoniae and 9 isolates were E.coli. 20/32 (62%) isolates were from renal transplant patients and 12/32 (38%) were from CHD patients. 28/32 (87.5%) isolates harboured blaNDM gene with 16/28 (57%) isolates also harbouring a blaOXA48like gene. 4/32 (12.5%) isolates harboured blaOXA48like gene alone. 31/32(97%) isolates were positive for blaCTXMgp1 gene and 9/32(28%) isolates harboured blaCMY. 13/32 (40.6%) isolates were positive for the armA gene and 5/32 (15.6%) were positive for the mrtB gene. 27/32 (84.4%) isolates harboured the PMQR determinant-aac(6')-IB-cr and 26/32(81.3%) isolates harboured qnrB.

Conclusion: The high prevalence of CPE co-expressing other clinically relevant resistant genes from CHD and renal transplant patients substantiates the need for routine surveillance of CPE among chronic haemodialysis and renal transplant patients to prevent wide-spread dissemination of carbapenemases into the community.
Synthesis and biological evaluation of indole-based 2-Aryl-2,3-epoxy-1,4-naphthoquinones as methicillin-resistant staphylococcus aureus (MRSA) inhibitors

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Background: Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most successful human pathogens responsible for causing a wide range of infections ranging from Toxic shock syndrome to boils and endocarditis. MRSA has demonstrated extreme ability to resist antibiotics, thus necessitating a continuous search for new scaffolds active against it. Fosfomycin, a clinically utilized antibiotic for the treatment of MRSA infections, is known to act through covalent modification of biological thiols with its epoxide functionality. 2,3 epoxy-1,4-naphthoquinones are found to be constituents of numerous natural products. These compounds are found to be reactive with cellular thiols and generate reactive oxygen species (ROS), which can act to induce oxidative stress. Thiols, such as cysteine and glutathione are important for the maintenance of redox homeostasis in cells and hence, depletion of thiols could induce cellular stress and trigger cell death. Thus, such compounds might have therapeutic potential against MRSA infections.

Methods & Materials: We synthesized a library of indole-based 2,3 epoxy-1,4-naphthoquinones, varying the substitution on the epoxide carbons, and studied their reactivity with thiols, such as cysteine and glutathione. The synthesis of these compounds was undertaken in five steps from commercially available indole-3-carboxaldehyde.

Results: These compounds showed potent activity against drug-resistant clinical isolates of S. aureus including VRSA with MIC ranging from 0.06-0.12 mg/L. A good correlation was found between thiol-mediated decomposition profiles and MIC values of these compounds. The selectivity index of these compounds was >200, thus indicating specificity for bacterial cells and were found to be non-haemolytic against human RBCs. These compounds exhibited concentration dependent bactericidal activity with a ~5 log killing at 24 h in comparison to drug free control. These compounds exhibited a potent post-antibiotic effect when compared to Vancomycin. In order to elucidate their mechanism of action, resistant mutants with a MIC of 64 mg/L were generated (frequency ~10^-7) and are being characterized at the molecular level to decipher mechanism of action of these compounds.

Conclusion: A series of 2-Aryl indole-based 2,3-epoxy-1,4-naphthoquinones have been synthesized with potent anti-MRSA activity. These compounds potentially deplete thiols, thus enhancing ROS in bacteria, which might help in overcoming drug resistance.
Susceptibility pattern of healthcare-associated methicillin resistant staphylococcus aureus to Vancomycin and Daptomycin

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Background: Healthcare-associated methicillin resistant *Staphylococcus aureus* (HA-MRSA) is a major pathogen. Vancomycin is used in the treatment of serious infection caused by HA-MRSA. However, the emergence of Vancomycin intermediate *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA) is a matter of concern. The present study was conducted to determine minimum inhibitory concentration (MIC) of vancomycin and daptomycin among HA-MRSA in our healthcare settings.

Methods & Materials: A total of 110 HA-MRSA isolates (as defined by Centres for Disease Control and Prevention criteria) were collected over a period of seventeen months. Vancomycin MIC was determined by agar dilution method according to CLSI guidelines. Daptomycin MIC was determined by E-test (BioMerieux, France).

Results: Out of the total HA-MRSA isolated, 53.6% (59/110) had vancomycin MIC of 2µg/ml. Intermediate resistance to vancomycin was detected in 03.6% (04/110) of the HA-MRSA and these isolates had a vancomycin MIC of 4µg/ml. All HA-MRSA isolated were sensitive to daptomycin.

Conclusion: Occurrence of VISA among the HA-MRSA is a matter of concern. As these strains do not respond to vancomycin treatment hence their detection is crucial. VISA cannot be detected by routine disk diffusion method. Determination of MIC of vancomycin is necessary for the detection of VISA. Daptomycin can be effectively used in the treatment of infections caused by VISA isolates in our healthcare settings as resistance to this antibiotic has not yet been observed.
Prevalence and antibiotic sensitivity of Staphylococcus aureus and Pseudomonas aeruginosa in middle ear fluids of chronic suppurative otitis media and chronic rhinosinusitis patients undergoing ear surgery

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Background: Chronic Suppurative Otitis Media (CSOM) and Chronic Rhinosinusitis (CRS) are strongly associated common diseases with a significant impact on people’s quality of life worldwide. Emergence of antimicrobial resistance of the causative microbes poses problem in management of the disease. The present study aimed to find the microbial prevalence and compare the antibiotic sensitivity in middle ear fluid isolates of CSOM and CRS patients with CSOM in South Indian population.

Methods & Materials: 86 subjects with CSOM and 68 patients with CRS undergoing ear surgery at a MAA ENT Hospitals, Hyderabad, South India between 2009 and 2015 were included in the study. The middle ear aspirates were collected aseptically, cultured by conventional methods and tested for antibiotic sensitivity using Kirby Bauer disc diffusion method. Chi-square analysis was performed to test the difference between the two groups.

Results: The present study included 99 males and 57 females with mean age of 34.06 ± 19.215yrs. Significant difference in prevalence of bacterial isolates with respect to sex and age was seen between the two groups (table 1). The most frequent microbial isolates in CSOM subjects was Pseudomonas aeruginosa (24%) followed by Staphylococcus aureus (19%) whereas in CRS with CSOM subjects, Staphylococcus aureus was 45% and Pseudomonas aeruginosa was 20%. Antibiotic susceptibility of staphylococcus aureus was high to cefotaxime, amikacin and gentamicin in both the groups. Antibiotic resistance of staphylococcus to ciproflaxacin is 78.8 % and vancomycin is 55 % in CSOM subjects . High rate of antibiotic sensitivity of Pseudomonas aeruginosa was observed for imipenem, piperacillin tazobactam, cefotaxime and amikacin in both the subgroups. Antibiotic resistance of Pseudomonas aeruginosa to ciproflaxacin is 55%, Gentamicin 47.2% and Cefepime 50% in CSOM subjects. Antibiotic resistance of Pseudomonas aeruginosa was not seen in CRS with CSOM subjects (Table 2).

Conclusion: The antibiotic sensitivity of the common microbes differed significantly between CSOM and CRS with CSOM subjects in South Indian Population. The present study warrants the need for evaluation of antimicrobial susceptibility profile of the causative microbial pathogens before administration of antibiotics to treat CRS with CSOM in particular.
The emergence of cotrimoxazole and quinolone resistance in Shigella sonnei

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**Background:** The emergence of cotrimoxazole resistance has been a dominant and consistent character in our isolates of Shigella sonnei. To study the behaviour of these emerging strains and characterise mechanisms of resistance to cotrimoxazole & ciprofloxacin the following study was performed.

**Methods & Materials:** Isolates of *Shigella sonnei* confirmed by standard methods from 2012 to 2015 were subjected to antimicrobial susceptibility testing using the Kirby Bauer method as per Clinical laboratory standards institute and PCR for the detection of virulence genes. The degree of relatedness between the isolates was assessed by ERIC PCR followed by gel image analysis. Dendrogram was generated using Pyelph. PCR was carried out to determine the mechanisms of resistance to cotrimoxazole and ciprofloxacin.

**Results:** Of 34 *Shigella sonnei* isolates, cotrimoxazole resistance was common (94.1%) followed by ciprofloxacin (47%). Majority carried the *ipaH* gene (97%) followed by *ial* (17.6%), *sen* (11.7%), *set 1 & set2* (5.8%). No *stx* element was found. ERIC–PCR analysis of the isolates resulted in four major ERIC groups labelled Eric group I,II,III and IV. Type III was the dominant (44.1%) type. Majority harboured *dhfr1* (94.1%), *sul2* (85.2%) followed by *sul3* (55.8%), *sul1* (11.7%). Two isolates that were resistant to cotrimoxazole were negative for the *sul* genes but harboured the *dhfr1* gene. All the phenotypically ciprofloxacin resistant isolates (47%) were positive for presence of *gyr A, gyr B, parC and parE*. Also, *qnrB* was the most prevalent PMQR gene (93.7%) while, *qnrC* was positive in 18.7% of isolates. None were positive for *qnrA and qnrS*. Two (0.1%) of the isolates were positive for *aac(6\')-lb* gene. The *qepA* gene regulating the efflux pump was negative in all the isolates studied. One isolate that was susceptible to all antibiotics tested negative for all the genes.

**Conclusion:** The emergence of *Shigella sonnei* with a characteristic sulphonamide resistance needs to be addressed further in detail and the increasing trend of resistance to quinolones is a point of concern. This study also shows the emergence of a particular ERIC type in the background of this evolving resistance pattern.

**Conclusions**
Carriage of multiple gene cassettes mediated extended spectrum cephalosporinase within diverse incompatibility (Inc) plasmid groups among gram negative rods in a tertiary referral hospital of India

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Background: Extended spectrum β-lactamases pose to be major health problem in hospital settings worldwide. Infection with ESBL producing organisms result in poor clinical outcome, overdue initiation of suitable antibiotic treatment, longer hospital stays and greater hospital operating cost. Management of treatment against these strains become complicated when the resistant determinants are associated with integron and horizontally transferable due to their location within plasmid. In this study, we report multiple gene cassettes mediated extended spectrum cephalosporinase within diverse Inc plasmid groups among gram negative rods for the first time in India.

Methods & Materials: A total number of 458 clinical isolates of gram negative rods were collected during November 2011 to October 2013 from Silchar Medical College and Hospital. ESBL status was detected by phenotypic screening as per CLSI criteria and multiplex PCR assay followed by sequencing. Genetic environment was determined by integrase gene PCR and location of blaESBLs within gene cassette was investigated by 59base element PCR and sequencing. Plasmid transferability was done by transformation and conjugation while incompatibility profiling was done by PCR based replicon typing. DNA finger printing of isolates was done by ERIC and REP PCR.

Results: A total of 56 isolates were found harboring blaESBLs by PCR and sequencing. All of them were carrying class I integron and blaESBLs was found to be located within gene cassette and conjugative plasmid. Further, PCR based replicon typing established presence of diverse Inc plasmid types viz. FIA, FIB, P, FrepB, K, B/O, I1/Iγ and Y. The isolates showed high MICs against cephalosporins (≥256 µg/ml), and monobactam (≥256 µg/ml) but was found in susceptible range against Ertapenem drug. The isolates were found clonally unrelated.

Conclusion: The study revealed presence of gene cassette mediated blaESBLs among gram negative rods within hospital environment. Presence of blaESBLs in diverse Inc plasmid groups suggests their diverse source of acquisition. Current study insists vital requirement for regular monitoring of these resistant determinants to execute the right antibiotics policy so as to reduce the irrational use of expanded spectrum cephalosporins and to decrease the antibiotic pressure and treatment failure in clinical setting in this part of the world.
Beta-lactamases in a Nepalese hospital: Wake up before the "biological quake" destroys you

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Background: In this era of modern medicine, antimicrobial resistance has emerged as a major health catastrophe. Emergence of drug resistance mechanisms like Extended-spectrum beta–lactamases (ESBLs), AmpC beta-lactamases and metallo-beta-lactamases (MBLs) can be regarded as "biological quake" posing therapeutic challenge to the health care settings. Therefore, this study was designed to determine the prevalence of ESBL-, MBL-, AmpC-producing bacteria in hospital-admitted patients.

Methods & Materials: A prospective study was conducted among the inpatients of Medicare National Hospital in central Kathmandu for four months (April-July, 2015), a period when the hospital was engaged with "Nepal Earthquake 2015" victims too. Different clinical specimens were collected, processed and the isolates were identified following standard methodology. Antibiotic sensitivity test was done by Kirby-Bauer disc diffusion method. ESBL was detected by standard combination disc method. Besides, tests for ESBL, AmpC, and co-production of ESBL and AmpC were done by MASTDISCS™ ID AmpC and ESBL Detection Discs, as well as ESBL and AmpC detection Ezy MIC™ Strip (HiMedia, India). EDTA-Imipenem combination disc method was followed for MBL detection.

Results: Among the total 75 gram-negative bacterial isolates resistant to third generation cephalosporin, ESBL was seen in 30.6% (n=23). Similarly, MBL and AmpC production were seen in 8% (n=6) and 1.3% (n=1) respectively. Interestingly, ESBL-AmpC co-production was found in 4% (n=3). Escherichia coli was the most frequent ESBL-producer (n=20). E. coli was found to produce MBL (n=4), AmpC (n=1), and ESBL-AmpC combination (n=2) as well. Two isolates of P. aeruginosa were ESBL-AmpC co-producers. Out of 23 ESBL-producer, 78.2% (n=18) were from intensive care unit patients. The ESBL-producing bacteria showed sensitivity to different antibiotics as follows- meropenem (n=21, 91.3%), amikacin (n=20, 86.9%), and cefoperazone-sulbactam (n=19, 82.6%). Consistent results were found with different methods employed for detection of ESBL and AmpC.

Conclusion: ESBL-producing bacteria were more commonly seen though AmpC- and MBL-producers were relatively less frequent. Special strategy like antibiotic stewardship should be followed in our setting before the situation turns out to be havoc. Identification, characterization and surveillance of antibiotic susceptibility profile of beta-lactamase-producing organisms can lead to successful infection control.
Fecal carriage of carbapenem resistant enterobacteriaceae (CRE) and risk factor analysis in hospitalised patients: A single centre study from India
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Background: Carbapenem resistant Enterobacteriaceae (CRE) have emerged and disseminated widely causing a variety of infections. The emergence of carbapenem resistant Enterobacteriaceae is associated with limited therapeutic options and increased mortality in patients infected by these strains. These organisms also have the propensity to undergo widespread dissemination via mobile genetic elements. Enteric strains possessing these carbapenemases have shown remarkable success in the form of large scale geographical dissemination. Gut colonization by CRE may act as reservoir of these pathogens for dissemination within an enclosed setting as in a hospital. To the best of our knowledge, there are no studies of CRE fecal carriage using genotypic methods and those analysing risk factors leading to such colonization in hospitalised patients in India.

Methods & Materials: We conducted the present study to observe gut carriage rate of CRE in patients presenting to our tertiary care hospital using both phenotypic (modified Hodge test) and genotypic (polymerase chain reaction for bla\textsubscript{VIM}, bla\textsubscript{KPC}, bla\textsubscript{IMP} and bla\textsubscript{NDM-1} genes) methods and tried to identify the risk factors for CRE gut colonization.

Results: A total of 239 fecal swabs yielded 259 Enterobacteriaceae isolates, of which 108 isolates (majority included E. coli and Klebsiella spp.) from 84 patients showed presence of CRE (prevalence 84/239; 35.14%); 28 isolates from 23 patients had bla\textsubscript{NDM-1} while 20 isolates from 17 patients possessed bla\textsubscript{VIM} gene. No isolate was positive for bla\textsubscript{KPC} and bla\textsubscript{IMP} genes. Although highest isolation of CRE was from the wards, approximately half of patients of intensive care units yielded CRE in fecal swabs. The CRE were also found to have significantly high antimicrobial resistance as compared to non-CRE isolates. Multivariate analysis of risk factors showed use of any antibiotic (P=0.002), cephalosporins use (P=0.000) and presence of any indwelling device (P=0.014) as independent risk factors for acquiring gut colonization.

Conclusion: The study is the first from India to show high CRE carriage in patients admitted to a tertiary care centre and emphasises the need of strict antimicrobial stewardship implementation in hospitals to prevent dissemination of multidrug resistant CRE.
Molecular mechanisms of efflux pump mediated resistance in clinical isolates of multidrug resistant Pseudomonas aeruginosa

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**Background:** Intrinsic resistance in *Pseudomonas aeruginosa* is largely due to overexpression of efflux pumps, among which members of the Resistance Nodulation cell Division family (RND) viz., MexAB-oprM, MexXY-OprM, MexCD-OprJ & MexEF-OprN are predominant. MDR infections are difficult to treat with antibiotics and hence combinations of antibiotics with efflux pump inhibitors (EPIs) have been proposed. Studies on efflux mediated resistance among MDR are lacking. Therefore the present study is aimed to determine the effect of efflux pump inhibitor and overexpression of efflux pumps in clinical isolates of *P. aeruginosa*.

**Methods & Materials:** A total of 213 *P. aeruginosa* clinical isolates were collected and antimicrobial susceptibility testing (AST) was done by standard techniques. Forty MDR isolates were chosen and included for the present study. EPI test was carried out by determining minimum inhibitory concentration (MIC) of antibiotics with and without EPI (Phe-Arg β-naphthylamide) at a concentration of 50µg/ml. Reporter antibiotics used were carbenicillin(MexAB-OprM), erythromycin(MexCD-OprJ), norfloxacin(MexEF-OprN) and gentamicin(MexXY-OprM). In the case of 21 isolates, expression of efflux pump encoding genes (*mexA*, *mexC*, *mexE*, *mexX*) was determined by quantitative Real Time PCR with *rpoD* gene as endogenous control. Wild-type PAO1, PAOΔmexR::Gm·, PAONB, PAOΔEF, PAOΔmexZ::Gm were used as reference strains.

**Results:** EPI test showed 4-64 fold MIC reduction in 23/40(58%) isolates for carbenicillin and erythromycin and in 32/40(80%) isolates for norfloxacin. No MIC reduction was observed in 32 (80%) isolates for gentamicin whereas one isolate showed 64-fold reduction. Quantitative analysis revealed that on comparison with PAO1 strain, expression of *mexA* and *mexE* increased by 3-10 and 2-3 fold respectively in all isolates. *mexC* expression ranged from 5-24 fold increase in 14(67%) isolates, 73-294 fold and 630-fold in 4 and 1 isolate respectively. One isolate each showed 2 and 17 fold difference in *mexX* expression whereas no expression was observed for the remaining isolates.

**Conclusion:** The present study documents the prevalence of efflux pump mediated resistance among MDR isolates of *P. aeruginosa*. All MDR isolates over-expressed at least one of the efflux pumps. Among the RND family genes studied, *mexC* showed a wide range as well as high levels of expression indicating its role in multidrug resistance in *P. aeruginosa*. 
Identification of erm and msrA genes in inducible clindamycin resistance of clinical isolates of staphylococcus aureus by polymerase chain reaction and D-test in Iran

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Background: Resistance to macrolide can be mediated by erm and msrA genes in Staphylococcus aureus. There are the evidences that show erm genes may be causative agent of inducible or constitutive resistance. D-test as a simple phenotypic test can differentiate between S. aureus isolates with inducible clindamycin resistance. The aim of this study was to investigate the incidence of inducible clindamycin resistance and determine the most frequency of erm and msrA genes among S. aureus isolates.

Methods & Materials: In this study a total of 124 non duplicate isolates of S. aureus collected from different clinical specimens were tested with disk diffusion method. All of isolates were tested by PCR for mecA, ermA, ermB, ermC and msrA genes.

Results: According to PCR results 48.4% had mecA gene and 51.6% were mecA negative. By phenotypic D-test method, 32.3% revealed inducible resistance and recorded as D and D⁺. Sensitive and constitutive phenotypes were found in 54.8% and 12.9% of isolates respectively. Inducible clindamycin resistance was more prevalent in MRSA (29%) than MSSA isolates (2.4%). Among studied erm genes, the most frequency genes were ermA and ermC with 41.1% and 17.7% respectively. Three isolates of them had D phenotype, while the PCR results of erm genes were negative. All isolates were negative for ermB or msrA genes.

Conclusion: Since S. aureus isolates with inducible resistance may mutate and change to constitutive resistance, so for prevention of treatment failure, we suggest that inducible resistance test be performed in the isolates which are resistant to erythromycin and sensitive to clindamycin.
An issue of Public Health concern due to emerging drug resistance against Toxascaris leonina (Linstow, 1909) in Asiatic lions (Panthera leo persica)

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Background: *Toxascaris leonina*, an ascarid nematode having wild carnivores as definitive hosts, possesses significant zoonotic potential as larvae of the parasites have been found invading laboratory animals and cases of human invasion had also been reported. Due to the ability to infect the incidental hosts, the professionals dealing with the carnivores including veterinarians and zoo keepers are at constant risk of exposure to the parasite, leading to *visceral larva migrans* in the victims.

Methods & Materials: The faecal samples of Asiatic lions, kept at MC Zoological Park, Chhatbir, Punjab, India were screened seasonally (from January 2013 to September 2014) at regular basis for any parasitic infestation by using floatation-concentration and sedimentation techniques. Morphological (classical parasitological) and molecular confirmation (polymerase chain reaction targeting internal transcribed spacer regions) of the eggs retrieved was carried out. Therapeutic intervention was carried out thrice by using routinely used benzimidazole compound, fenbendazole (@ 10 mg/ kg body weight, once daily for three days for the first time followed by same dose rate but with extended period dose schedule (5 days) for the second time) and a macrocyclic lactone, ivermectin (@ 100µg/ kg body weight), during third management schedule.

Results: The ascarid eggs recovered were delineated as *Toxascaris leonina* eggs based on morphometric and molecular studies. Therapeutic management with fenbendazole @ 10 mg/ kg body weight, once daily for three days proved ineffective with maximum faecal egg count reduction on day 3 post treatments (69.35%), which further reduced to 51.61% at day 21 post treatments. Therapeutic intervention with extended period dose schedule (5 days) with fenbendazole (@10 mg/ kg body weight) although proved effective and showed a maximum faecal egg count reduction of 95.34% at day 7 post treatments. But, ivermectin proved effective as a faecal egg reduction of 95.74% was recorded at day 7 post treatments.

Conclusion: Present study highlights the molecular confirmation of *T. leonina* and first case of fenbendazole resistance against *T. leonina* in Asiatic lions, which could be considered as alarming as resistance against such parasites may result in increased vulnerability of human beings (persons in contact) for infection. Thus, alternate drug management study needs to be followed.
Prevalence of plasmid-mediated quinolone resistance determinants in enterobacteriaceae strains in West-Iran

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**Background:** The quinolone groups are an important class of broad-spectrum antimicrobial agents. Plasmid mediated quinolone resistance (PMQR) determinants have emerged as a significant concern in recent years. This study reports on screening of resistant-isolates to fluoroquinolone antimicrobial agents for PMQR determinants and detection of \( qnr \) and \( aac\)\((6')\)-\(Ib-cr \) genes.

**Methods & Materials:** A total of 100 fluoroquinolone-resistant *Enterobacteriaceae* were isolated from 3 hospitals in Hamadan, west provinces of Iran, from October 2012 to June 2013. The isolates were identified by biochemical tests and confirmed by PCR. Antimicrobial susceptibility to 14 antimicrobial agents including levofloxacin and ciprofloxacin was determined by disk diffusion methods and ciprofloxacin MIC was obtained by broth microdilution method as Clinical Laboratory Standards Institute (CLSI) recommendations. The isolates were screened for the presence of \( qnrA \), \( qnrB \), \( qnrS \) and \( aac\)\((6')\)-\(Ib-cr \) genes using PCR assays.

**Results:** Among the screened isolates, 64 strains (64%) of *Escherichia coli*, 23 strains (23%) of *Klebsiella pneumoniae*, 13 strains (13%) of *Proteus mirabilis* were collected as quinolone-resistant isolates. Out of 100 isolates, two (2%) were positive for \( qnrS \), seventeen (17%) isolates were positive for \( qnrB \) and we did not find \( qnrA \) gene in any of the isolates. There were also 32 positive isolates for \( aac\)\((6')\)-\(Ib-cr \) determinant.

**Conclusion:** We described the prevalence of \( qnr \) and \( aac\)\((6')\)-\(Ib-cr \) genes in fluoroquinolone-resistant *Enterobacteriaceae* in Hamadan city. The carriage rate of multidrug-resistant *Enterobacteriaceae* in healthy people in Hamadan City is extremely high. Moreover, genes encoding transferable quinolone resistance, in particular \( aac\)\((6')\)-\(Ib-cr \), are highly prevalent in these strains.
Background: Blood stream infections are an important cause of mortality and morbidity. Illness associated with blood stream infection ranges from self-limiting infections to life-threatening sepsis that require rapid and aggressive antimicrobial treatment. So, knowledge of local pathogens and susceptibility patterns is essential to start prompt and appropriate empirical therapy and also to formulate and update antibiotic policy. Increasing rates of antimicrobial resistance, changing patterns of antimicrobial usage, and the wide use of indwelling catheters may change the epidemiology and outcome of bloodstream infection.

Methods & Materials: The data from blood cultures received over a period of 4 years from 2011-2014 were retrospectively analysed by using WHONET 5.6 software. Common demographic parameters of patients were noted. The change in trends of etiology and susceptibility pattern of pathogens causing BSIs at a tertiary care hospital was studied.

Results: A total of 12553 blood cultures were processed with 1651 (13.1%) showing positive cultures. Maximum blood cultures were received from medical wards followed by paediatric ICU. Gram negative bacteria (GNB mainly Enterobacteriaceae) were predominant cause of bacteremia in all 4 years but pseudomonas and acinetobacter were emerging as newer pathogens. Predominant isolate in 2011 was *E.coli* (44%) and in 2014 was *Acinetobacter spp* (88%). We have observed increase in multidrug resistant bacteria over 4 years. The prevalence of ESBLs has increased from 61.6% (2011) to 66% (2014) and that of Carbapenamase producers from 13.6% to 25%. MRSA has jumped from 50% to 60% and Amp C producers were detected at a rate ranging between 69-71%. The most effective antimicrobials against GNB were carbapenems and aminoglycosides and against gram positive cocci were Vancomycin and Linezolid.

Conclusion: Gram negative bacteria were predominant cause of bacteremia. Drug resistance in bacteria is increasing over the years which need effective implementation of the antibiotic policy formulated according to local susceptibility pattern.
Emergence of multidrug resistant and non-vaccine serotypes of streptococcus pneumoniae in a tertiary care hospital, Southern India

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**Background:** *Streptococcus pneumoniae* is an important human pathogen causing invasive and non invasive infections associated with high rates of morbidity and mortality. Antimicrobial therapy and vaccines have been used to treat and control these infections. Against this background, it becomes necessary to monitor antimicrobial susceptibility and the circulating serotypes of *S. pneumoniae* to achieve optimum control.

**Methods & Materials:** All the clinical isolates of *S. pneumoniae* confirmed by lytA-targeted PCR collected in our laboratory from December 2014 to August 2015 were tested for their susceptibility to various antibiotics by disc diffusion following standard procedures (CLSI, 2014). MIC values of penicillin were determined by E-strips (Biomerieux, France). Serotyping was carried out by latex agglutination using grouped antisera (Statens Serum Institut, Poland) and confirmed by PCR.

**Results:** Thirty eight isolates were obtained from various clinical specimens including blood, CSF, sputum and pus. The following were the documented resistance rates to various antibiotics –erythromycin- 24%, clindamycin-13% of which 20% showed inducible resistance, tetracycline - 26% , levofloxacain - 11% and cotrimoxazole-45%. Two isolates, one each from CSF and sputum were resistant to penicillin with MIC values of 1.5µg/ml and 16µg/ml respectively. No resistance was observed to vancomycin, ceftriaxone and linezolid. Multi drug resistance was seen in 21% of isolates with a majority showing resistance to macrolides, tetracylines and cotrimoxazole. The penicillin resistant strain from sputum of an adult with bronchiectasis was of 47A/F serotype, which is not covered under PCV13 or PPV23. Three other non- vaccine serotypes, 33D (from lens tissue )  31 and 19C (both from sputum of adults with lower respiratory tract infections) were also identified.

**Conclusion:** Emergence of both MDR and non - vaccine serotypes of *S.pneumoniae* is of grave concern and should prompt greater efforts at restricted antimicrobial use, particularly for upper respiratory infections as well as studies to enhance knowledge on circulating serotypes to improve vaccine efficacy.
Molecular serogrouping of serologically atypical shigella isolates from South India
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Background: Shigellae are the causative agents of bacillary dysentery, represents a significant public health problem worldwide especially in developing countries. New serotypes or subserotypes in Shigella are not uncommon and are reported from different parts of the world. Notably, atypical Shigella exhibit greater antibiotic resistance than typical Shigella serotypes and also found to harbour integrons which has the ability to acquire resistance genes. The purpose of this work was to amplify rfb regions (contains the genes coding for the enzymes responsible for O-antigen synthesis) of non-agglutinating Shigella isolates to characterize these by endonuclease restriction and to study the presence of antimicrobial resistance genes (AMR).

Methods & Materials: A total of 3647 faeces specimens were processed between January to December 2014, among these, 27.5% (n = 176) were identified as Shigella spp. Eight non-agglutinable Shigella isolates obtained were included in the study for molecular characterization. These strains were tested for their antimicrobial susceptibility against six antibiotics by Kirby Bauer disc diffusion method. All the isolates were screened for the AMR genes (dhfr1A, sulII, bla-OXA, bla-TEM, bla-CTX-M, AmpC and qnrA, B, S) by PCR. The isolates were also amplified for their O-antigen gene cluster using Expand long template PCR system and the amplicons were further restricted using MboI enzyme.

Results: The prevalence of shigellosis during the study period was 4.8%. The antimicrobial resistance for the atypical Shigella were 50% for ampicillin, co-trimoxazole (87%), nalidixic acid (37%), cefixime (12.5%) and all were susceptible to norfloxacin and cefotaxime. The AMR gene PCR results showed 25% of dhfr1A (n =2), 62.5% sulII (n =5), 50% bla-TEM (n =4), 50% qnrS (n =4) and all the isolates were negative for bla-OXA, bla-CTX-M and AmpC genes. rfb-RFLP results showed clearly identifiable and reproducible pattern, six different patterns were obtained.

Conclusion: This study revealed the description of O-specific patterns allowing serotype identification without the use of antisera. Although the number of atypical Shigella strains in this study was only eight, thorough and strict monitoring of isolation of such atypical strains would help to understand the actual disease burden caused by the new Shigella serovars and consequently to study the epidemiology of shigellosis.
Profile of genes coding for Carbapenemases among resistant acinetobacter species from a tertiary care centre: A laboratory based study

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Background: Acinetobacter species are being increasingly reported to be associated with infections among seriously ill individuals. These infections are difficult to treat due presence of genes coding for resistance to most of the available antibiotics. The study was conducted to identify the presence of commonly described genes coding for carbapenemases among the isolates.

Methods & Materials: The study was a cross-sectional laboratory based observational study from a tertiary care hospital. 89 consecutive (single isolate per patient) isolates of Acinetobacter species were included. Study period: isolates collected during 2011-2012 from clinical samples and molecular studies done in 2013. Organisms identified as Acinetobacter baumanii complex (Acb complex) using biochemical tests were included in the study. Antibiotic susceptibility test was done by Kirby Bauer Disc diffusion. Resistance to carbapenems was confirmed by Microbroth dilution. PCR was done to identify the presence of the following genes using published primers, Oxa(23like,24like,58,51like,NDM,VIM and IMP. ERIC PCR was done to identify if there was any clonal similarity among these isolates.

Results: The isolates demonstrated resistance to nearly all antibiotics tested except for Netilmicin for which 11%(10/89) were susceptible.

The samples from which the Acb complex were isolated: Respiratory secretions (n=60,67%), pus samples(n=25,28%), urine (n=10,11%) blood 2 and other fluids 3 isolates.

53,(60%) of the samples were from patients admitted to the intensive care unit.

The common mechanisms of Resistance for carbapenems were found to belong to the OXA carbapenemases(23 ) in 92% of isolates,followed by VIM(65%) .Around 82% also had AmpC betalactamases.NDM detected in 12.

ERIC PCR: Isolates showing >90% of similarity were grouped into 4 different clusters, genetic heterogeneity was noticed among each cluster.

Conclusion: Acinetobacter species though previously thought to be contaminants have now gained notoriety as multidrug resistant hospital acquired pathogens due to acquisition of a number of resistance genes. Genes coding for Carbapenem resistance identified commonly in our study were the OXA 23like and VIM.NDM was also detected. Considerable heterogeneity was noted by ERIC PCR.
Background and Methods & Materials

Results: All (100%) the strains harboured the quinolone resistance gene gyrB, while, gyrA and parE were present in 93.7% each, parC in 85.4%, aac(6′)-Ib and qnrS in 41.6% each, qnrB in 34.7%, qnrC in 31.2%, qepA in 18.7% and qnrA in 12.5%. Genes which are responsible for resistance to aminoglycoside aac(3)-IV (14.5%), aadB (6.2%), sulfonamides (sulI) (77%), (sulII) (31.2%), (sulIII) (6.2%), trimethoprim (dhfrI) (50%) and tetracycline (tetA) (56.25%), (tetY) (20.8%), (tetD) (18.7%), (tetE) (18.7%), (tetC) (16.6%), (tetB) (8.3%), chloramphenicol (catI) (39.58%) and β lactam (blaCTX) (64.5%), (blaTEM) (20.8%) and (blaSHV) (8.3%) were also widely distributed. Integrons were noted in 70.8% (int1), 33.3% (int3) and 31.2% (int2), while none harboured the macrolide resistance gene erm.

Results

Conclusion: A diverse pattern was observed with regards to antimicrobial resistance genes in DEC. This study also illustrates the importance of integrons in the epidemiology of antibiotic resistance in DEC strains in our setting.
Conclusion
Safety and therapeutic efficacy of staphylococcus aureus specific lytic phage against multidrug-resistant S.aureus (MDRSA) in BALB/c mice: A prospective study
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**Background:** The use of phage therapy as an alternative method of treating infections caused by multidrug-resistant bacteria has been a controversial issue. The present study sought to determine safety and therapeutic efficacy of environmentally obtained *Staphylococcus aureus* lytic phage against multidrug-resistant *S.aureus* (MDRSA) in mice.

**Methods & Materials:** Phages and MDRSA were isolated from sewage and waste water collected from within Nairobi County. The isolated *S.aureus* bacterium was screened for resistance towards; Ceftazidime, Oxacillin, Vancomycin, Netilmicin, Gentamicin and Erythromycin, Trimethoprim-Sulfamethoxazole and Cefuroxime. Thirty BALB/c mice were randomly assigned into three groups; the MDRSA infection group (n=20), the phage-infection group (n=5) and non-infection group (n=5). After 24 or 72 hours post-infection (p.i.) with MDRSA, the infected mice were either treated with a single dose of clindamycin (8mg/kg/bwt) or 108 PFU/ml of *S.aureus* phage or a combination (clindamycin and *S.aureus* phage). Safety was determined by monitoring animal physical health post-infection as well as gross pathology and histopathology. Bacteremia was determined daily for 10 days and used to establish therapeutic efficacy of the phage.

**Results:** Treatment with phage was efficacious (100%) compared to clindamycin (62.25%) at 24hrs p.i and 87.5% at 72hrs p.i.) while combination therapy (75% at 24hrs p.i. and 100% at 72hrs p.i.). Efficacy of the treatment regimens was dependent on the time of treatment post infection. The mice infected with MDRSA and treated with phage had no bacteremia at day 7 post-treatment compared to those treated with clindamycin and combination therapy (P < 0.001). There were no tissue abscesses, inflammation in the brain, lungs and liver tissues of phage treated mice compared to those treated with clindamycin and combination therapy.

**Conclusion:** The *S.aureus* phage obtained from sewage and waste water from within Nairobi County was safe and possessed therapeutic efficacy against MDRSA bacterium.
Molecular characterization of high level aminoglycoside resistant non-urinary isolates of enterococcus species

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**Background:** The optimal success of bactericidal therapy against Enterococcal infections currently relies on the use of the synergistic combination of a cell wall-active agent, a penicillin or a glycopeptide, with an aminoglycoside (gentamicin / streptomycin). Nonetheless, the acquisition of ribosomal mutations and/or aminoglycoside modifying enzymes (AGMEs) had led to the emergence of high-Level aminoglycoside resistant strains that pose a significant therapeutic challenge. Hence, the study was aimed to detect the incidence of HLR among the non-urinary Enterococcal isolates.

**Methods & Materials:** Twenty six non-urinary isolates of Enterococcus species (E. faecalis (n=15), E. faecium (n=5), Enterococcus spp (n=6)) isolated from pus (n=20), fluids (n=5) and blood (n=1) were included in the study. Susceptibility to vancomycin (30µg), teicoplanin(30µg), linezolid(30µg), gentamicin(120µg), streptomycin(300µg) and screening for HLR was performed as per CLSI, 2015. Molecular detection of the genes encoding vancomycin, aminoglycoside resistance viz., vanA, vanB, aac(6')-Ie–aph(2")-Ia, aph(2")-Ib, aph(2")-Ic, aph(2")-Id, aph(3")-IIIa and ant(4')-Ia was carried out by multiplex PCR.

**Results:** High-level resistance to gentamicin and streptomycin (HLG R HLSR) was observed in 14 (53.8%) isolates while, 6(23.1%), 2(7.7%) isolates exhibited HLGR HLSR and HLGS HLSR phenotype respectively. MIC of gentamicin and streptomycin of the HLR, HLSR isolates was found to be > 500 µg/ml and >2000 µg/ml respectively. aac(6')-Ie–aph(2")-Ia and aph(3")-IIIa were detected in 5(22.7%) and 1(4.5%) of the HLR isolates respectively while, 9(40.9%) of the HLR isolates harboured both aac(6')-Ie–aph(2")-Ia and aph(3")-IIIa. None of the isolates exhibited resistance to teicoplanin and linezolid. Nevertheless, one isolate exhibited intermediate resistance to vancomycin by disc diffusion but was confirmed to be susceptible by E-test and did not harbour vanA and vanB.

**Conclusion:** Aminoglycosides are the most frequently prescribed antibiotics in clinical practice as they possess good pharmacokinetics and exhibit synergism with beta-lactams and glycopeptides. However, we report an increased incidence of HLR among the non-urinary Enterococcal isolates. This is indicative of an increased dissemination of aminoglycoside resistance genes in our geographical setting. Hence, prompt detection and characterization of HLR strains is essential as these have lost synergism with the cell wall active agents. Also, absence of VRE could be attributed to the restricted usage of vancomycin in our setting.
Tracking trends in antibiotic effectiveness using the Drug Resistance Index

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Background: Antibiotic resistance is a major global health threat. Owing to the multiplicity of bacterial pathogens and drugs to treat them, quantifying the overall status of antibiotic effectiveness or resistance is challenging. We used the Drug Resistance Index (DRI), a novel metric which aggregates antibiotic consumption and resistance into a single measure, to compare antibiotic effectiveness by country and time.

Methods & Materials: Adapting the methodology described by Laxminarayan and Klugman (2011), we calculated annual DRIs for 30 countries. Our index includes six common bacterial pathogens and antibiotic classes used for their treatment. The pathogens included are: Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecium, and Enterococcus faecalis. The antibiotic classes are: aminoglycosides, broad-spectrum penicillins, carbapenems, cephalosporins, narrow-spectrum penicillins, and quinolones. Antibiotic use frequencies were obtained from IMS Health. Data on antibiotic resistance were obtained from ResistanceMap (resistancemap.cddep.org). To calculate the index, we first multiplied the frequency of use of each antibiotic class by the aggregated resistance rates of pathogens against which the antibiotic class is used. Then, we summed the weighted resistances to each antibiotic class and generated DRI values.

Results: We present the DRI values as ranging between 0 and 100, where 0 represents maximum antibiotic effectiveness and 100 indicates no effectiveness of antibiotics. In 2010, among 29 countries, the highest DRIs were observed for India (74.4), Greece (45.5) and Portugal (43.4), while the countries with the lowest DRIs were Sweden (3.0), Denmark (8.2) and Norway (8.5). Among the 18 countries for which data were available to calculate DRIs for the entire 2000-2010 period, increases in DRIs were observed in 15 countries. Czech Republic had the greatest increase of 24.6 points (4.0 in 2000 to 28.6 in 2010). The United Kingdom had the greatest decline of 10.3 points (42.0 in 2000 to 31.7 in 2010). Across these 18 countries, the DRIs increased by an average of 7.2 points during 2000-2010, implying that the overall effectiveness of antibiotics declined.

Conclusion: The DRI can be a valuable tool to quantify, track and compare antibiotic effectiveness worldwide.
Concentration dependent carbapenem exposure alters the plasmid copy number within nosocomial isolates of Escherichia coli harboring bla\textsubscript{NDM-1}: A study from Northeast India

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**Background:** Expansion of bla\textsubscript{NDM-1} and its broad host range results in the propagation of this resistance determinant within different incompatibility group of plasmids. It is crucial to understand how bacterial extra chromosomal entity responds in the pathogen encoding the resistance gene when they are exposed to carbapenem antibiotics. This study investigates the change in plasmid copy number of bla\textsubscript{NDM-1} under the pressure of different concentration of carbapenem antibiotics within nosocomial isolates of *E. coli* from a tertiary referral hospital of northeast India.

**Methods & Materials:** Clinical isolates of *E. coli* harboring bla\textsubscript{NDM-1} within three different incompatibility plasmid groups viz; Inc F, Inc A/C and Inc K were grown under the exposure of imipenem, meropenem and ertapenem with concentration of 0.5, 1, 2 and 4µg/ml for each antibiotic. Plasmid was extracted for each sample and quantitative real time PCR was carried out to estimate the relative copy number of bla\textsubscript{NDM-1} and fold change was normalized against a housekeeping gene *rpsel*. Curing of different Inc type plasmid was done by treating the isolates with different concentrations of curing agents viz. sodium dodecyl sulfate, acridine orange and ethidium bromide.

**Results:** It was observed that the copy number of bla\textsubscript{NDM-1} gene within Inc F and Inc A/C type of plasmids was consistently higher as the concentration of imipenem and meropenem was increased whereas in case of ertapenem it was vice versa. In Inc K type of plasmid, the copy number of bla\textsubscript{NDM-1} was consistently raised only under the exposure of meropenem while with imipenem and ertapenem, the copy number got reduced. Plasmids encoding bla\textsubscript{NDM-1} could be successfully eliminated after single treatment with SDS and is considered as the most efficient curing agent. The cured mutants became susceptible to carbapenems, quinolones and to aminoglycosides antibiotics.

**Conclusion:** The study could establish that different carbapenem concentrations can alter the plasmid copy number irrespective of their incompatibility types. Also, it underscores stability of different incompatible plasmids when exposed to a curing agent. Thus, the current investigation is helpful in predicting the vertical transmission dynamics of resistance determinants, which will be key for infection control and slowing down expansion of multidrug resistance in hospital settings.

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Background: Bloodstream infections (BSI) cause important morbidity and mortality worldwide. In Cambodia, there are limited data on pathogens causing BSI and their antimicrobial resistance. We describe the cumulative results of a prospective blood culture surveillance study in an adults’ hospital in Phnom Penh, Cambodia between July 2007 and December 2014.

Methods & Materials: Blood cultures (2 x 10ml) were performed in adults presenting with systemic inflammatory response syndrome (SIRS) at Sihanouk Hospital Centre of HOPE, Phnom Penh, Cambodia. Isolates were identified using standard microbiological techniques; antibiotic susceptibilities were assessed using disk diffusion and MicroScan, with additional E-test, D-test and double disk test, according to CLSI guidelines.

Results: A total of 16,815 pairs of blood-bottle samples from 12,092 patients yielded 1,467 clinically significant organisms (CSO, 8.8%). Among them, 1160 (n= 79.1%) were Gram-negative bacteria, comprising Escherichia coli (n= 436; 29.7%), Salmonella (n= 291, 20.0%); Burkholderia pseudomallei (n= 133, 9.0%) and Klebsiella spp. (n= 98; 6.7%). Among E. coli, we noted combined resistance to amoxicillin, trimethoprim-sulfamethoxazole and ciprofloxacin in 256 (58.7%) isolates; 59.0% (190/322) isolates tested were extended spectrum beta-lactamase (ESBL) producers, resistance to third generation cephalosporins increased from 50.0% in 2008 to 73.2% in 2014). In 2 patients, carbapenem-resistant Klebsiella pneumoniae was isolated. For Burkholderia pseudomallei, no resistance towards the drugs of use (i.e. ceftazidime, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid) was observed. We noted an emergence of Salmonella Paratyphi A since mid-2012 which was still ongoing at the end of the study period. For Salmonella Typhi (and other Salmonella serotypes), there was a trend of increasing frequency of reduced susceptibility to ciprofloxacin; ESBL was observed in 2 out of 18 Salmonella Choleraesuis isolates.

Among Gram-positive CSO, Staphylococcus aureus (n= 121, 8.2%) was predominant; methicillin resistance was observed in 32/121 (26.4%) of S. aureus isolates and increased over time (15.4% versus 36.4% for the years 2008 versus 2014 respectively).

Conclusion: Sustained surveillance of BSI allows detection of clinically relevant resistance patterns and outbreaks. BSI in Cambodian adults is mainly caused by difficult-to-treat pathogens. Our findings have important implications for treatment guidelines and urge for microbiological capacity building and solid interventions to contain antibiotic resistance.
Antibiotic sensitivity patterns among ESBL UTIs in Sri Lanka
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Background: Extended-spectrum β-lactamase (ESBL) producing organisms causing urinary tract infections (UTI) are increasing in incidence and poses a major burden to health care requiring treatment with expensive antimicrobials and prolonged hospital stay. The prevalence of ESBL producing organisms particularly in the Asian region remains unknown. In a study carried out in a tertiary care center in India, 70/218 (32.1%) clinical isolates of Enterobacteriaceae were confirmed as ESBL. Of them K. pneumonia were the most common ESBL producers (46.4%), followed by E. coli (31.7%). Previous studies to evaluate antibiotic susceptibility shows high sensitivity to meropenem (95-100%) with aminoglycoside susceptibility ranging from 45-60%. Objective of this study was to evaluate the antibiotic sensitivity patterns of ESBL UTIs in Sri Lanka.

Methods & Materials: Patients with ESBL-UTI admitted to Professorial Medical Unit, Colombo North Teaching Hospital, Ragama over a period of 6 months from January-July 2015 were recruited to the study. Their Urine culture and ABST reports were analysed after obtaining informed written consent.

Results: There were 52 patients who consented for the study: males 30 (57.7%), mean (SD) age 64.11 (12.59) years. The most common organisms causing the ESBL-UTI were E. coli in 44 (84.6%), followed by Klebsiella in 8 (15.4%). The ESBL organisms were mostly sensitive to carbapenems; Meropenem 50 (96.2%) and Imipenem in 38 (73.1%). The other sensitivity patterns were Amikacin in 30 (57.7%), Nitrofurantoin in 24 (46.2%) and Ceftriaxone in 2 (3.8%). None were sensitive to Ceftazidime. Meropenem resistance was found in 2 (3.8%) and were E. coli. These two patients had received multiple antibiotics including meropenem in the recent past for recurrent UTI.

Conclusion: It is evident from the above data that Carbapenems remain as the first line therapy for the majority of UTIs caused by ESBL producing organisms in the local setting. However 3.8% prevalence of meropenem resistance among the study population should draw attention of clinicians and needs implementation of measures to prevent emergence and spread of carbapenem resistant ESBL organisms.
Background: This study was carried out to find out the average direct hospitalization cost and length of hospital stay for patients infected with Carbapenem-resistant Klebsiella pneumonia (CRKp) and compare it with that of patients infected with Carbapenem-sensitive Klebsiella pneumonia (CSKp).

Methods & Materials: A cross sectional study was carried out from January-December 2014 and the data for hospitalization cost was collected for the patients with CRKp and CSKp infections from the medicine ICU for 72 patients admitted to the hospital. The data was analyzed for the site of infection, length of stay and average direct hospitalization cost which was then compared between the two groups.

Results: During the study period, 101 patients were diagnosed with Klebsiella pneumoniae infection. 61.79% of the infections were respiratory, 24.52% urinary tract-related, 13.2% systemic and 0.47% skin and soft tissue-related. The mean age of the study population was 51.7 ± 15.7 years. The median length of stay for CRKp was 12 (11; 23) days as compared to 8 (5.2; 13.2) days in CSKp patients. The median direct hospitalization cost was calculated to be INR 43,274 (24,898; 16,0315) in case of CRKp versus INR 23,452 (12,489; 47,349) in CSKp patients.

Conclusion: It was observed that the average cost of overall therapy and the average no. of hospitalization days was higher in CRKp group compared to CSKp group. Antibiotic resistance is a growing phenomenon and the resistance to Carbapenems can lead to increased burden of morbidity and treatment cost for patients.
Development, optimization, standardization and validation of a simple in-house agar gradient method to determine vancomycin MIC’s for Staphylococcus aureus

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Background: MIC determination has became easier, with introduction of the E-test. The point of contention has been the cost of the commercial strips which has restricted its use in resource limited countries. We attempted to develop, standardize and validate a simple in-house agar gradient method, to determine MIC of vancomycin for clinical isolates of S. aureus.

Methods & Materials: In-house strips were made from Whatmann filter paper no 1. The strips were impregnated with varying concentration of vancomycin solution so as to create an increasing antibiotic gradient along the strip. During the standardization step, MIC’s of 90 clinical strains of S. aureus and ATCC 29213 were tested by the broth microdilution and commercial strip followed by the in-house strip. The results were kept blinded during the development stage. Variables for the preliminary considerations for optimization and standardization of the In-House Agar Gradient strip were considered to correct the outliers. This was followed by validation stage where MIC’s of 90 different clinical strains of S. aureus and ATCC 29213 were determined by the in-house and results were kept blinded. This was followed by determination of MIC’s by broth microdilution and commercial strips. An MIC reading of ± 1log2 dilution compared with broth microdilution was considered as an outlier

Results: During the optimization and standardization stage there were 7/90 outliers in the clinical strains and no outliers seen with the ATCC 29213 control strain. Corrective action was performed by increasing precaution during the antibiotic solution impregnation stage. During the validation stage, only 4/90 outliers were observed in the clinical strains. The commercial strips had 29/90 among clinical and 15/30 outliers in the control strain during the prevalidation phase. The supplier was informed to maintain cold chain and during the validation phase the outliers for commercial strip were 18/90 and 4/30 in the clinical and control strain respectively.

Conclusion: Vancomycin sensitivity is reported as MIC. Expensive commercial strips can be replaced by in-house strips using this simple technique after validating it with a gold standard method like broth microdilution.
Multidrug-resistant acinetobacter baumannii – plasmid-borne carbapenem and aminoglycoside co-resistance causing outbreak in Southwest Virginia

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Background: Multidrug-resistant (MDR) Gram-negative bacterial pathogens, often resistant to more than one class of antibiotics including carbapenems and aminoglycosides, pose serious threats in healthcare settings worldwide. A 2015 White House task force issued guidelines to combat antibiotic-resistant strains in the United States, which the Center for Diseases Center has termed “nightmare bacteria”. MDR pathogens cause at least two million illnesses and 23,000 deaths annually in the U.S. MDR A. baumannii (MDR-Ab) cause 10% of hospital-acquired infections (HAI) with 70% patient mortality. A mini-outbreak of carbapenem-resistant (CR) MDR-Ab (CR-MDR-Ab) occurred during a 2009-2010 H1N1 epidemic at Carilion Medical Center (CMC) in Virginia, U.S; three of the nine patients died. To develop effective strategies for prevention, control and treatment of MDR infections, we are performing whole genome analysis of the clinical isolates of CR-MDR-Ab.

Methods & Materials: To date, we have analyzed 68 Ab clinical isolates, including five CR-MDR-Ab outbreak strains. We have sequenced and analyzed whole genomes of the following MDR-Ab isolates: an isolate from outbreak patients (CMC-MDR-Ab4); a carbapenem-sensitive isolate (CMC-MDR-Ab59); and a CR-MDR-Ab (CMC-MDR-Ab66) isolate from sporadic cases. The sequencing was performed on the PacBioRSII platform.

Results: The CR CMC-MDR-Ab strains were found to carry two plasmids, pCMCVTAb1 and pCMCVTAb2. The latter is conjugative type and carried two transposons: Tn2008-like, containing a beta-lactamase gene (blaOXA23) and conferring CR, and a TnaphA6 element causing aminoglycoside resistance and further reducing treatment choices. Their chromosomes carried five bla genes and an aphA1 gene. A PCR analysis based upon the resistant determinants showed that all outbreak isolates (100%) carried pCMCVTAb2 containing the two transposons. Fourteen of the remaining 63 isolates (22.22%) carried pCMCVTAb2 and of these, six (42.85%) carried Tn2008-like, and two (14.28%) carried TnaphA6. These differences are statistically significant using Fisher’s exact, two-tailed p = 0.0011, p = 0.0445 and p = 0.0018.

Conclusion: The results suggested that pCMCVTAb2 was responsible for the CR-MDR outbreak. A PCR analysis based on the antibiotic-resistant genes of pCMCVTAb2 could be used for rapid identification of CR-MDR-Ab strains, thereby helping to prevent the spread and providing guidance for identification and treatment of hospitalized patients with CR-MDR-Ab infections.
Biofilm formation by Staphylococcus species on exposure of sub-lethal concentration of vancomycin

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Background: Staphylococcus is responsible for community acquired and nosocomial infection in different sites of human body. Drug resistant Staphylococcus such as Methicillin resistant Staphylococcus (MRSA) and vancomycin resistant \textit{Staphylococcus aureus} (VSRA) has become common concern. The drug resistance increases 10 to 1000 folds when there is production of biofilm. This study has assessed level of biofilm production without and on exposure to sublethal concentration of vancomycin against the clinical isolates of staphylococcus.

Methods & Materials: A total 103 pure growth of staphylococci isolated at Department of Microbiology, Tribhuvan University Teaching Hospital (TUTH), Kathmandu, was included in the study. Of them 25 were MSSA, 25 MRSA, 23 MS-CONS and 30 MR-CONS. All isolates were subjected for determination of MIC of vancomycin by following the standard methods (Creasten \textit{et al} and modified by Stepanovic \textit{et al}, 2007)

Results: Among 103 isolates of \textit{Staphylococcus} species, many (97.1\%) were found to have MIC of vancomycin within susceptible range, with few (2.9\%) were found to have MIC of intermediate range. For \textit{Staphylococcus aureus} 24.0\% had MIC level of 2mg/l and for CONS 45.2\% had the MIC level of $\geq 2$mg/l showing high number of isolates towards upper limit of susceptible range of Methicillin resistant isolates.

Among the isolates, 63.1\% of \textit{Staphylococcus} species were producing different degree of biofilm with CONS sharing the larger percentage. Sub-lethal concentration of vancomycin has significantly (p<0.05) induced biofilm formation in both MRSA and MSSA, however, induced effect seems to be higher in MRSA isolates. On the other hand, in cases of CONS, sub-lethal concentration of vancomycin could not show significant induced effect (p>0.05)

Conclusion: This study has concluded that the MIC value of clinical isolates for Staphylococcus is increasing and the sublethal dose of Vancomycin induces biofilm production and thereby producing VRSA. Therefore the MIC determination prior to therapy and proper dosing should be done.
Influence of variation in the sequence(s) of factors essential for methicillin resistance (fem genes) on the expression of resistance to Lysostaphin and secretion of DNAse.

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Background: In *S. aureus*, cell wall consists of pentaglycine bridge which is catalyzed through *fem* (factors essential for methicillin resistance) genes namely *femX*, *femA* and *femB*. *FemX* adds the 1st glycine, *femA* adds the 2nd and 3rd glycine and *femB* adds the 4th and 5th glycine of the pentaglycine bridge. The target of the lysostaphin is the pentaglycine cross-bridge between 3rd & 4th glycine. Mutations in any one of these *fem* genes, reportedly confer lysostaphin resistance but decreases resistance to beta-lactam compounds such as methicillin and oxacillin.

Methods & Materials: We screened all *Staphylococcus aureus* isolates (n=100) for expression of DNAase and screened 45 isolates which showed variations in *fem* gene sequences for the sensitivity of Lysostaphin. We determined the lysostaphin (20ug) susceptibility through broth dilution method (Hardy’s Test) and DNAse by both methyl green agar method and also by PCR.

Results: Those *S.aureus* isolates which failed to amplify any of the *fem* genes, considered as total *fem* mutants displayed 80% resistance to lysostaphin when compared with wild isolates. Mutations in *femA* alone resulted in 70%, 52% (*femB*) and 52.7% (*femX*) resistance to lysostaphin. *Fem* mutants which showed variable expression of DNAse were (*femA* 2%; *femB* 5%, *femX* 2%) on DNAase agar, although all of these isolates were positive for DNAase PCR. When compared between the species specific markers ie; *nuc* gene and *fem* (A,B and X genes), *nuc* gene proved to be reliable for species identification.

Conclusion: Lysostaphin cleaves the inter-glycine peptide bond between the 3rd and 4th glycine and it is expected that any variation in the *fem A* or *femB* gene or *fem X* may have an impact on the synthesis of the pentaglycine bridge in the cell wall of *S.aureus*. Our results demonstrate that mutations in *fem* genes not only affect sensitivity to lysostaphin but also other cell wall functions like secretion of DNAse. We will also present results of analysis of these *fem* mutants on expression of PBP2a and the sensitivity to various antibiotics.
Lower respiratory tract infection in two tertiary hospitals of Kolkata and carbapenem resistance


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Background: Lower respiratory tract infection (LRTI) is a significant cause of morbidity and mortality worldwide. Surveillance studies documented increasing occurrence of infection by resistant organisms posing challenge to management and control policy. Carbapenem-resistant Enterobacteriaceae (CRE) are a serious threat to public health. Present work is aimed to show the susceptibility pattern in Enterobacteriaceae and non-fermenting gram negative bacilli (NFGNB) causing LRTI and selective molecular characterization of Carbapenem resistant isolates.

Methods & Materials: The study was conducted in hospitalized LRTI patients of two urban teaching hospitals (January 2013 - December 2013). Bacterial isolates were identified from validated sputum, endotracheal aspirate, bronchoalveolar lavage fluid and pleural fluid (n=231) by standard culture method. Antimicrobial susceptibility and minimal inhibitory concentration values were determined. The clinical breakpoints for Meropenem were: susceptible ≤1.0 mg/L, intermediate 2.0–3.0 mg/L, and resistant ≥4.0 mg/L. The same for Ertapenem were: ≤0.5 mg/L, 1.0 mg/L, ≥2 mg/L respectively. MIC value for Ertapenem ≥0.5 was set as screening breakpoint to detect carbapenemase for Enterobacteriaceae. Following Imipenem MIC breakpoints were adopted to indicate MBL production: Pseudomonas aeruginosa, ≥4µg/mL; Acinetobacter spp and other NFGNB other than P. aeruginosa, >2µg/mL. Phenotypic evaluation for detection of beta lactamases (ESBL, Amp C) and carbapenemase (KPC, MBL) were done in Ertapenem/Imipenem non-susceptible isolates. Ertapenem/Imipenem non susceptible Enterobacteriaceae and NFGNB were subjected to molecular characterization of KPC/MBL (NDM) determinants. Clinical profile of patients harbouring these genes were analysed and compared.

Results: Total Enterobacteriaceae and NFGNB (n=88) included Klebsiella pneumoniae (n=47), Escherichia coli (n=9), Enterobacter spp. (n=3), Citrobacter freundii (n=1), Pseudomonas aeruginosa (n=20) and Acinetobacter spp. (n=8). Tigecycline and Colistin were the most effective antimicrobials followed by Carbapenems. Total Carbapenem resistant isolates were n=28(32%) of which 9(32%) were detected harbouring bla<sub>NDM</sub>. No bla<sub>KPC</sub> was detected. Significant association of bla<sub>NDM</sub> carrying isolates in LRTI patients with neurological abnormality was observed (Cerebrovascular accident patients; P value 0.025). LRTI patients with Carbapenem resistant isolates harbouring bla<sub>NDM</sub> gene did not have increased mortality.

Figure 1: Isolates identified in two hospitals and their (%) of Carbapenem resistance

Figure 2: Resistance profile of K.pneumoniae and P.aeruginosa isolate(%) tested against broad spectrum antimicrobials

Conclusion: Evidence of substantial percentage of Carbapenem resistant isolates carrying bla<sub>NDM</sub> gene in the present clinical setting indicates need for effective and rapid infection control measures.
Characterization of multidrug-resistant escherichia coli and salmonella isolated from food producing animals in Northeastern India

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Background: Unraveling the determinants of multidrug resistance (MDR) in food animals is either poorly reported or scarce from northeastern India. Given the propensity of low and middle-income countries towards unprecedented global resistance hotspots, the present study aims to characterize MDR Escherichia coli and Salmonella isolated from food producing animals in this part of the country, a region of strategic significance.

Methods & Materials: A total of 668 faecal samples randomly collected from swine, cattle, goat, yak and avian scattered in Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Sikkim were analysed. Antimicrobial susceptibility test against 20 antimicrobials were done including determination of MIC values for Salmonella isolates. The confirmed Salmonella (n=47) and MDR E. coli (n= 174) were subjected to molecular characterization for MDR genes (n=19). In-vitro transfer of resistance genes and efflux-pump gene expressions (marA, mdfA) were also checked for the MDR isolates.

Results: Out of 453 samples screened for E. coli, 386 (85.2 %) isolates was recovered, while 47 (7.03 %) Salmonella was isolated from 668 samples examined. Above 6 percent E. coli showed resistance to all the tested antibiotics. Over 80 percent Salmonella were resistant to amikacin, cefazolin and tobramycin and other remaining exhibited high frequency of resistance against cefuroxime (74.5 %) and gentamicin (68.1 %). Overall marA gene was the most common type with 15 percent harborring the gene. Above 10.8 percent isolates possessed sul1, sul2, tetA, strA genes and above 5 percent carried tetB, adaA, armA, blaTEM and blaCTX-M, however, blaShv gene, armB and rmtC were not recovered. Plasmid mediated quinolone resistant gene positive strains totaled to 4.4 (qnrB) and 2.07 (qnrS) percent while qnrA was not detected. Species and region-wise distributions were variable. Resistant traits were transferable and the resistant isolates had shown ≥2 fold increase in the expression level of marA.

Conclusion: Emergence of MDR in E. coli or Salmonella among the animal populations of NE India was evident from the study revealing above 85 percent MDR isolates. These results emphasize the urgent need for surveillance of antibiotic resistance incorporating rational and regulated use of antibiotics in Livestock farming.
Correlation of carbapenem resistance and hypermucoviscosity in K.pneumoniae isolated from blood culture at a tertiary hospital in South India

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Background: Carbapenem resistant K.pneumoniae (CRKp) are posing an increasing threat to treatment of infections especially in children and immunocompromised patients. Recently there are cases with hypervirulent/hypermucoviscous K.pneumoniae (hvKp) infections associated with high mortality rates of 52% and metastatic spread. Hence it is important to know the factors contributing to disease severity in hvKp infections. There are limited reports regarding carbapenem susceptibility of hvKp which however is an important factor contributing to patient outcome. We aimed at studying the prevalence of genes coding for carbapenamases among hvKp and distribution of meropenem MIC among hvKp and non-hvKp.

Methods & Materials: A total of 77 CRKp isolated from blood culture during 2014 and 2015 at department of Clinical Microbiology, CMC, were included. Screening for carbapenem resistance was done by disc diffusion method for susceptibility to imipenem and meropenem and resistant isolates were included which were then subjected to E-test for meropenem. The results for antimicrobial susceptibility testing were interpreted according to CLSI guidelines. The resistant isolates were then subjected to string test which is the phenotypic test for hvKp, and multiplex PCR for detection of genes for carbapenemase production.

Results: Among the 77 CRKp isolates tested, 25 (32%) were string test positive and 52 (68%) were negative. The majority of hvKp, 10 (40%), co-expressed NDM and OXA48-like genes while 19 (37%) non-hvKp expressed OXA48-like. Meropenem MIC range obtained was 0.064 µg/ml - >32 µg/ml. 72% and 65% of hvKp and non-hvKp had MIC of ≥32 µg/ml. Overall, OXA48-like genes were the most predominant genes isolated from 25 (32%) isolates tested.

Conclusion: Co-expression of NDM and OXA48-like genes might contribute to the increased MIC for meropenem among the hvKp which is a potential threat for patient management. Monitoring the frequency of isolation and susceptibility profile for hvKp will help in achieving clinical cure by administering the right antibiotic and prevention of metastatic spread of infection. Decreased susceptibility and the hypermucoviscous nature might contribute to the severity of infection and increased mortality in infections with hvKp than the classical K.pneumoniae.
Prevalence and antibiogram of pseudomonas aeruginosa isolated from clinical specimens in a Teaching Hospital, Kathmandu
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Background: Pseudomonas aeruginosa is a leading cause of nosocomial infections. Increased resistance in this organism continues to pose a significant threat to patient care because of limited therapeutic options. Knowledge of the prevalence of P. aeruginosa in various infections and their antibiotic susceptibility pattern is of utmost importance for selection of appropriate therapy. Objective of the present study was to determine the prevalence and resistance pattern of P. aeruginosa isolated from various clinical specimens to Imipenem and other commonly used antibiotics in Nepal Medical College and Teaching Hospital (NMCTH), a teaching hospital in Kathmandu, Nepal.

Methods & Materials: P. aeruginosa isolated from various clinical specimens like pus, sputum, blood, urine, catheter tips (devices), renal stones and body fluids processed in the clinical laboratory, department of Microbiology, NMCTH were included in the study. Isolation, Identification and antimicrobial susceptibility pattern were performed using standard microbiological techniques.

Results: A total of 102 isolates of P. aeruginosa were evaluated. The prevalence rate of the organism was 5.1%, out of which 75 (73.5%) were from inpatients and 27 (26.5%) were from outpatients departments. Urine and sputum yielded highest number of the isolates 37 (36.3%) each followed by pus and devices 10 (9.8%) each. Highest percentage of the organism 36 (35.3%) was isolated from the patients who were of more than 60 years of age. Nineteen (18.6%) of the organisms was seen to be multi-drug resistant. The organism showed maximum resistance to Piperacillin (57.1%) followed by Ciprofloxacin (36.7%), Ofloxacin (28.8%) and Gentamycin (30.9%). Only 6.5% of the isolates were resistant to Imipenem.

Conclusion: The antibiotic susceptibility pattern of bacterial pathogen like P. aeruginosa in the hospital settings should be continuously monitored and the results readily made available to clinicians so as to maximize the possibility of administering an effective therapeutic agent whenever needed.
Risk Factors for acquisition of invasive infections with NDM-1+ K. pneumoniae
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Background: Carbapenem resistance conferred by New Delhi metallo-β-lactamase 1 (NDM-1) is an increasing global public health problem and defining risk factors for acquisition of infections with NDM-1 containing organisms is urgently needed for its control.

Methods & Materials: We investigated the patient and hospital related risk factors for acquisition of in patients at CMC Hospital infected with invasive Klebsiella pneumoniae with and without NDM-1. This was a retrospective case-control study of patients admitted at CMC Hospital, Vellore between Oct 2009 and Sept 2014, with blood culture + invasive Klebsiella pneumonia that were NDM-1+ (case), NDM-1 ESBL+ (ESBL+ control), or NDM-1 ESBL− (ESBL− control). Cases and controls were matched on date of admission ± 45 days.

Results: There were 101 NDM-1+ cases, and 100 ESBL+ and 101 ESBL− controls, with little difference in mean ages: 31.20±22.94, 29.78±23.30, and 41.55±21.11, respectively. NDM-1+ subjects were more likely to have received antibiotics in the last 180 days than either the ESBL+ (OR= 1.478 [0.756-2.887]; P= .253) or ESBL− (OR= 1.713 [0.863-3.402]; P = 0.124) controls, and were more likely to have acquired the infection nosocomially than the ESBL+ (OR= 1.653 [0.861-3.174]; P = 0.131) and ESBL− (OR= 3.390 [1.808-6.329]; P = <0.001). NDM-1+ patients were more likely than ESBL+ (OR =2.114 [1.202-3.719]; P = 0.01) and ESBL− (OR =1.910 [1.088-3.352]; P = 0.024) to have been admitted to the ICU. The Case Fatality Ratio (CFR) was significantly higher in NDM-1+ patients than in ESBL+ (2.26; P <0.001) and ESBL− (3.8 P <0.001). The mean length of hospital stay for NDM-1+ patients was 31.30±31.827, and was significantly higher in than in ESBL+ (23.21±17.252; P = 0.001) and ESBL− (19.68±18.55; P =0.002).

Conclusion: NDM-1+ K. pneumoniae invasive infections are as likely to be acquired in the community as nosocomially but in the latter circumstance are more likely to be acquired in the ICU. The CFR of 52% is twice that of ESBL+ invasive Klebsiella.
Assessment of antibiotic resistance patterns of the fecal coliforms isolated from Cauvery River and screening of novel herbal lead molecules against probable drug targets of MDR pathogens by computational virtual screening

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Background: The present study focuses on elucidation of microbial pollution and antibiotic resistance profiling of bacteria isolated from River Cauvery. This study focused to screen herbal leads against probable drug targets of multidrug resistant isolates by computational virtual screening.

Methods & Materials: Water samples were collected during 2011-12 from ten hotspots. The physiochemical characteristics and microbial counts were determined. Kirby Bauer disc diffusion assay was used to investigate the antibiotic sensitivity profile of various isolates. Selected virulent toxins and gene products for bacterial drug resistance were identified as the probable drug targets. The 3D structures of these were predicted by homology modeling. The drug likeness and pharmacokinetic properties of selected phytoligands were computationally predicted. The binding properties of ligands and drug targets were studied by molecular docking.

Results: The samples collected from all the hotspots showed high bacterial count ($P<0.01$). Out of 848 isolates, 93.51% (n=793) demonstrated multidrug resistance to most antibiotics. 96.46% (n=273), 93.85% (n=107), 94.49% (n=103), 90.22% (n=157) of the isolates were exhibited multi-drug resistance to 30, 32, 40, 37 antibiotics and they were identified to be *E.coli*, *Enterobacter cloacae*, *Pseudomonas trivialis* and *Shigella sonnei* respectively. The prevalence of *blaTEM* in all the four isolates and *dhfr* in *E.coli* and *Shigella sonnei* were identified. *Yokenella*, *Morganella*, *Citrobacter*, *Serratia*, *Salmonella*, *Proteus*, *Klebsiella*, *Edwersiella*, *Alcaligenes*, *Staphylococcus* and *Vibrio* were also showed multidrug resistance. This study suggested Cadinane and Cedrol showed good binding against shiga and cholera toxins respectively. Violaxanthin identified as therapeutic lead for hemolysin-E. Afzelin and gallocathecin were identified as lead against *blaTEM* and *dhfr*. Baicalein and Luteolin were identified as leads against *aph* of *S.typhi*. Resveratrol and Wogonin were identified as leads against *dhfr* of *Salmonella typhi*. Herniarin and Pyrocide were identified as leads against *dfrA1* of *Vibrio cholerae*. Taraxacin and Luteolin against *mec1* and Apigenin and Luteolin against *vanH* of *Staphylococcus aureus* as therapeutic leads.

Conclusion: The current study showed predominance of many multidrug-resistant bacteria in River Cauvery. Bioinformatics study suggests that phytoligands present in various herbs exhibit promising binding activities against the drug targets of multidrug resistant strains.
Antibiotic use increases risk of acquiring ESBLs and Enterobacteriaceae resistant to ciprofloxacin in a prospective cohort of Dutch travelers.

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Background: Travel to foreign countries is a well-known risk factor for acquiring resistant strains, especially of significance for low prevalence countries. In this study we investigated the prevalence and risk factors for travel-related acquisition of ESBL-producing Enterobacteriaceae (ESBL-E), ciprofloxacin resistance and carbapenemases.

Methods & Materials: Pre- and posttravel data (a questionnaire and rectal swab) were collected from 445 participants, age ≥ 18 years. Data from 418 persons were included in the ESBL analysis and from 400 persons in the ciprofloxacin resistance analysis (the remaining persons carried already ESBL or ciprofloxacin resistant Enterobacteriaceae before travel and were excluded). Swabs were cultured with an enrichment broth containing ampicillin and subcultured on selective agar plates for ESBL detection (EbSA ESBL screening agar; Cepheid Benelux, Apeldoorn, The Netherlands), and on plates with a ciprofloxacin disc. ESBL production was confirmed with the double disc synergy test with clavulanic acid. Species identification and susceptibility testing were performed with the Vitek-2 system (Biomérieux). All isolates were subjected to ertapenem Etest. ESBL and carbapenemase genes were characterized by PCR and sequencing (BaseClear).

Results: Twenty-seven out of 445 travelers (6.1%) and 45/445 (10.1%) travelers had already an ESBL-positive or a ciprofloxacin-resistant strain before travel. Of the remaining travelers, 130/400 (32.5%) acquired a ciprofloxacin-resistant strain and 98/418 (23.4%) became ESBL-positive. Of the 98 ESBL-positive isolates, predominantly Escherichia coli and predominantly blaCTX-M-15, 20% was resistant to gentamicin, ciprofloxacin as well as co-trimoxazole. Multivariate analysis showed that travelers to Asia were significantly at higher risk for ESBL and ciprofloxacin resistance acquisition. Also travelers with diarrhea combined with antimicrobial use were significantly at higher risk for acquisition of resistant strains. One carbapenemase (OXA-48) producing strain was found in a participant after a visit to Egypt; however, the number of travelers visiting North Africa was low (n=12).

Conclusion: Travelling to Asia and travelers with diarrhea combined with antimicrobial use is an important risk factor for the acquisition of Enterobacteriaceae resistant to cephalosporins and quinolones and, at present rarely, carbapenems. Because travelers’ diarrhea is usually self-limiting, our findings implicate that caution is needed regarding the routine use, but also the choice of antibiotics for empirical therapy during travel.
Incidence of penicillin resistant streptococcus pneumoniae in sputum among children and elderly pneumonic patients attending two major hospitals in Khartoum

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Background: The incidence of pneumococcal disease is particularly high among young children and the elderly. *Streptococcus pneumoniae* is one of the major causative agents. The emergence of antimicrobial resistance threatens the successful treatment. Penicillin resistance has been encountered with increasing frequency in strains of *Streptococcus pneumoniae* from around the world. We noticed that there are cases which were difficult to treat among patients attending two major hospitals in Khartoum State.

Methods & Materials: Two hundred samples of sputum were collected from children (5-18 years) and elderly (above 60 years) (100 hundred from each group). The samples were collected from patients diagnosed clinically as having pneumonia, attending two major hospitals in Khartoum. Standards methods were used for identification and determination of susceptibility to antibiotics.

Results: The presence of the microorganisms was more in samples that was collected from children (60%) and (40%) in elderly patients. *Streptococcus pneumoniae* had been isolated from 40 samples (20%), 80% of the samples showed either no growth or growth of other organisms. Complete resistance to penicillin was detected by using oxacillin discs in (7.5%) of the isolates of *Streptococcus pneumoniae*. (12.5%) of the samples showed intermediate resistance and (80%) of the samples were sensitive to oxacillin.

Conclusion: The prevalence of penicillin-resistant *S. pneumoniae* in Khartoum is high (7.5%), although it is lower than that which had been reported in other some African and Asian countries. Effective practical methods for controlling microbial resistance to penicillin need to be developed.
High rate of antimicrobial resistance in bloodstream infections among infants and children in India

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Background: Surveillance of local microbiological profile and antimicrobial resistance pattern is essential to guide empiric antimicrobial therapy and establish strategies to deal with changing antimicrobial resistance. We aimed to study the profile of pediatric bloodstream infections, antimicrobial resistance and clinical outcomes in a tertiary care hospital in India.

Methods & Materials: Ambulatory and hospitalized children who had at least one blood culture between November 2012-April 2015 were included in this analysis. BACTEC BD9120 automated system was used for culture and antimicrobial sensitivities were assessed by the Kirby-Bauer method. Resistance was determined as per the CLSI 2014 guidelines. Multi-drug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial categories. Positive cultures obtained from ambulatory patients or within 72 hours of hospital admission were classified as community-acquired infections; those positive beyond 72 hours of hospitalization were healthcare-acquired infections.

Results: Among the 27,491 neonates and children evaluated during the study period, 12,264 blood cultures were obtained, with 882 positive cultures yielding 895 pathogens (culture positivity rate 7.3%). Isolates identified as commensals as per CDC guidelines (n=372) were excluded from the analysis. 55.8% (n=292) of the infections were community acquired. Hospital acquired infections were more prevalent among infants (<1yr) (n=109, 54.5%). Gram-negative organisms predominated (72.6% vs 25.0% of gram positive organisms and 2.4% fungi). Overall, 79.3% (n=415) of the isolates were resistant to at least one antibiotic while 45.3% (n=237) were multi-drug resistant. Extended spectrum beta-lactamase production was seen in 22.0% (n=110) of the isolates. Among the 47 isolates of Staphylococcus aureus, 20 (42.5%) were methicillin resistant. Hospital acquired infections were more frequently associated with multi-drug resistance than community acquired (64.0% vs 30.4%, p<0.001). The overall mortality rate was 10.8%. Children with multi-drug resistance had longer duration of hospitalization (17 vs 5 days, p=0.007) and were at greater risk of death (OR=3.04, p<0.001) than those without antimicrobial resistance.

Conclusion: High rates of antimicrobial resistance were seen in our study. The higher prevalence of drug resistance in hospital-acquired infections highlights the need for robust infection control practices. The study also reveals significantly longer hospital stay and higher risk of mortality in children with multi-drug resistance.
Investigating the distribution of integrons among clinical isolates of acinetobacter baumannii and their association to carbapenem resistance

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Background: Background: To determine the dissemination of classes of integrons and their roles in carbapenem resistant strains of *Acinetobacter baumannii*. Integron1, 2 and 3 have been reported in various microorganisms and are implicated in conferment of multi drug resistance. Carriage of carbapenem resistance genes on these integrons has not been consistent. Multidrug drug resistance is being increasingly reported in *A.baumannii*. Though integron 1 and 2 have been detected, there is no report from India correlating carbapenem resistance to the presence of integrons in *A.baumannii*.

Methods & Materials: Materials and methods: One hundred fifteen (n=115) clinical isolates of *A.baumannii* were collected from different clinical specimens of clinical microbiology laboratories two different tertiary care hospitals. Cultures were identified by routine microbiology methods and also by VITEK-2 system. Antimicrobial susceptibility testing and minimum inhibitory concentration for panel of 20 antibiotics were examined. To investigate classes of integrons, multiplex PCR for integrase *intI1* and *intI2* was performed along with *A.baumannii* species specific marker (Ab-ITS). ClassA, B and D carbapenemases were identified in these isolates and the distribution of carbapenemases with specific reference to type of integron has been determined by PCR.

Results: Results: A total of 115 isolates were included in this study. Genotypic and phenotypic results showed 93(80%) as carbapenem resistant and 22(20%) as carbapenem sensitive isolates. Out of the 93 carbapenem resistant isolates, 76 (81%) isolates possessed integrons. 58(62%) isolates carried class 1 integron and 18(19%) isolates carried class 2 integron. Surprisingly, 11 carbapenem resistant isolates and two carbapenem sensitive isolates harboured both classes of integrons. One carbapenem sensitive isolate had only class 2 integron.

Conclusion: Conclusion: Multiplex PCR for the detection and identification of integron classes along with species specific marker (Ab-ITS) will be a useful method for the epidemiological studies. This rapid, reliable determination of the genetic relatedness of clinical isolates in this antibiotic era is essential when investigating cases of nosocomial outbreak.
In-vitro assessment of antibiotic combinations against multidrug resistant gram negative bacilli in South India: A save carbapenem campaign

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Background: Infections caused by MDR pathogens are a therapeutic challenge being resistant to most of the available antibiotics and carbapenem mono-therapy is often used to treat these infections. Because resistance to carbapenems is increasing, there is a need to explore the potential of combination antibiotic therapy. As clinical data to support the choice of antibiotic combinations are sparse and conflicting, antibiotics in combination regimen are selected based on intuition and anecdotal reports and this approach is poorly guided, as the antibiotic combination selected may not always be optimal, because different combinations may be associated with different killing activity against MDR pathogens. This study aims to determine in-vitro efficacy of antibiotic combinations devoid of carbapenems, which will reveal the potential synergy between two antibiotics belonging to different chemical classes, especially when resistance to any one of them is present. Outcome of this study is expected to help in drafting robust in-house antibiotic policy and ease the pressure off carbepenems which are currently used overwhelmingly in clinical practice.

Methods & Materials: This is a prospective, experimental study where antibiogram of 85 MDR-GNB isolated from clinical samples was analysed. Minimum Inhibitory Concentration (MIC) of ceftazidime, amikacin, imipenem and ciprofloxacin for these bacteria was determined by broth micro-dilution according to CLSI guidelines. In-vitro effect of antibiotic combinations: CAZ- AMK, CAZ-CIPRO, IMP-AMK and IMP-CIPRO was studied by checker-board assay.

Results: 62.35%, 27.05% and 44.70% of these MDR-GNB were ESBL, AmpC and MBL producers respectively. 27.05% co-produced multiple β-lactamases. MIC$_{90}$ ranges for Ceftazimine: 16-≥1028µg/ml, Amikacin: 0.25-≥256µg/ml, Ciprofloxacin: 0.25-128µg/ml and Imipenem: 0.125-512µg/ml. CAZ- AMK and IMP-AMK combinations showed synergistic effect in 80%-90% of MDR-GNB with FICI value of ≤0.5. Higher rates of indifference (0.5 > FICI ≤2) and antagonism (FICI > 2) were observed with combinations having fluoroquinolones.

Conclusion: In-vitro antimicrobial activity of antibiotic combinations having 3rd or 4th generation cephalosporin with aminoglycosides was comparable to that of imipenem mono-therapy or combination and could be used to treat severe infections caused by MDR-GNB. Therefore combinations devoid of carbapenems should be advocated to prolong the clinical usefulness of this antibiotic group.
Prevalence of plasmid-mediated quinolone resistance genes among ciprofloxacin-resistant clinical isolates of Enterobacteriaceae over four years: A descriptive study

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**Background**: Plasmid-mediated quinolone resistance (PMQR) has received considerable attention recently. Data analysis in JIPMER revealed 85% of the Enterobacteriaceae isolates to be ciprofloxacin-resistant in 2012. Few reports regarding the prevalence of PMQR are available from India. Hence, the present study was carried out to ascertain the prevalence of PMQR genes among ciprofloxacin-resistant clinical isolates belonging to family Enterobacteriaceae.

**Methods & Materials**: A total of 790 Enterobacteriaceae clinical isolates resistant to ciprofloxacin by disk diffusion and agar dilution were studied. The isolates were screened for PMQR genes, and the positive isolates were further tested for the integrons intI1 and intI2. The hospital’s annual consumption data for fluoroquinolones were retrieved from JIPMER Pharmacy.

**Results**: Most strains exhibited high MIC values with 18% (150) showing MICs >256µg/mL. Overall, PMQR genes were detected in 525 (66%) isolates with 50 (6%) isolates carrying multiple PMQR genes. PMQR frequencies were as follows; aac(6')-Ib-cr 414 (64%), qnrB 97 (15%), and qnrS 64 (10%). Remarkably, 15 isolates were found positive for the single mutant variant of aac(6')-Ib that was confirmed by sequencing. The nucleotide sequences of the PMQR genes reported in our study are available in GenBank (KR080534 - KR080546). Variant alleles of PMQR genes were not found based on the pair-wise distance matrix analysis. None of the strains were positive for qnrA & qnrD. This study excluded qnrC allele. The frequencies of intI1 and intI2 among the PMQR isolates were 35% and 15% respectively whereas 7% had both the integrons. Transferability of PMQR genes to transconjugants was confirmed. Interestingly, neither the fluoroquinolone consumption in the hospital nor the frequency of PMQR isolates varied much in the four years of the study.

**Conclusion**: Only three strains with MIC >256µg/mL were PMQR-negative. The frequency of PMQR genes among the bacterial population studied is higher than reported elsewhere. Notably, all the ciprofloxacin-resistant *Escherichia coli* carried PMQR genes. The presence of PMQR gene in *Providencia rettgeri* has not been reported before. Finally, this study reports the single mutant variant of aac(6')-Ib gene for the first time from the clinical isolates. To conclude there is a need for rational usage of fluoroquinolones and reconsideration of their clinical breakpoints.
Towards a national action plan for antimicrobial resistance: The Kenyan experience

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**Background:** From the situation analysis on Antimicrobial use and resistance in Kenya 2011, 50% of the leading causes of death in Kenya are due to infectious diseases and antimicrobial resistance to microbes indicated rising trends of resistance to commonly used antibiotics (66%, 94%, 53%, 43% to cotrimoxazole, ampicillin, fluoroquinolones and penicillins respectively. This is attributed to inappropriate use of antimicrobials in humans and animals.

**Methods & Materials:** Desk review of recommendations made in minutes and reports of meetings, conferences and workshops on Infection prevention and control and AMR and publications from studies done in sentinel sites in Kenya.

**Results:** In 2009 the Global Antibiotic Resistance Partnership Kenya was established. GARP-K completed and launched a Situation analysis(SA) of antibiotic use and resistance in Kenya in August 2011; In November 2013-Kenya hosted the first Antimicrobial Resistance (AMR) awareness week and a regional antibiotic stewardship workshop in December 2013 whose recommendations were submitted to the National Infection Prevention and Control Committee (NIPCC) at the MOH in Kenya; Following this, findings of the SA were disseminated to health managers in 45 counties in Kenya, In March 2014 the NIPCC recommended the appointment of an AMR focal point; Through the Infection Prevention and Control Unit the National strategic plan for IPC with two strategic objectives specific for AMR: establishing a national AMR Surveillance system and the appointment of National Antimicrobial Stewardship Advisory Committee (NASAC);November 2014- during the 2nd National AMR week the Director of Medical Services established AMR program and appointed a multisectoral NASAC.

**Conclusion:** Low interest by leadership on AMR, lack of prioritization and resource allocation by the Ministry of Health for AMR related activities, changes in the system of governance in the country following devolution further slowed down the process of developing national strategies to fight AMR

The AMR program through the leadership of NASAC is in the process of developing a National Action Plan and policy for AMR addressing recommendations in the SA and leveraging the international health regulations and the global health security agenda. Through the AMR program, revision of the National IPC policy, guidelines and IPC training curriculum have included antimicrobial resistance and antimicrobial stewardship.
Antimicrobial use in children at a tertiary teaching hospital in New Zealand

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\textbf{Background:} Antimicrobials are one of the most common medicines prescribed for children. We aimed to describe the antimicrobial utilization in paediatric wards of a general teaching hospital and to assess the compliance of this pattern using three recently introduced guidelines: Christchurch Hospital local antimicrobial guideline, the Hospital Medicines List (HML – a national list of available pharmaceuticals) restrictions and the New Zealand National Formulary (NZF). The arrival of the Pharmaceutical Management Agency’s (PHARMAC) HML in July 2013 was the first introduction to a restrictive antimicrobial policy in New Zealand. NZF provides antibacterial policies which may indicate a range of drugs for general use, and permit other drugs only on the advice of the microbiologist or paediatric infectious disease specialist.

\textbf{Methods & Materials:} We conducted a prospective cross-sectional study of antimicrobial prescriptions over ten weeks (Oct to Dec, 2013). The audit included all paediatric wards at Christchurch Hospital, including medical and surgical patients and inpatients in a national oncology centre. We identified all children receiving antimicrobials and evaluated their prescription for dosage accuracy, reason of administration, duration of treatment, and compared these with both local and national guidelines.

\textbf{Results:} A total of 1000 antibiotic prescriptions were issued for 41 different antibiotics. Overall, 24\% (416 out of 1762) of the children received at least one antibiotic agent during their hospitalization. The most commonly prescribed antibiotics were amoxicillin/clavulanate, amoxicillin, trimethoprim/sulfamethoxazole, and flucloxacillin. Antibiotics were administered intravenously in 48\% of patients, and orally in 45\%. The most common reason for antibiotic prescription was surgical prophylaxis with 17\% of total prescriptions. Overall, there was 54\%, 80\%, and 61\% compliance with local antimicrobial guidelines, the Hospital Medicines List, and New Zealand Formulary respectively. Dosing errors were the most common reason for non-compliances.

\textbf{Conclusion:} Surgical prophylaxis made up the largest proportion of antimicrobial prescriptions and is an obvious target for any antimicrobial stewardship programme. The compliance to national guidelines for drug choices was acceptable but dosing errors still occur at high rates.
Antimicrobial prescribing patterns in a tertiary care hospital in India: Role of persuasive intervention for changing antibiotic prescription behavior
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Background: Antibiotic overuse is of great public health concern. Multiple studies performed globally have demonstrated a strong and consistent association between antibiotic use and antibiotic resistance (AMR), at both the individual and community levels. This study assessed whether intervention in the form of monthly feedback of antibiotic prescriptions and focus group discussions (FGD) could achieve a sustained decrease in antibiotic use in the surgical specialties.

Methods & Materials: This interventional study was performed at a tertiary care centre in New Delhi, wherein FGD was conducted with 35 surgical units from 13 specialties & assessed for the antibiotic usage during a 3 and 6 month time period post-intervention. The study included a 12 month pre-intervention period as a control. The main outcome measured was a change in antibiotic prescription rates reflected as daily defined doses per 100 bed days as defined by WHO.

Results: Reduction in level of antibiotic consumption was observed in 16 of 35 units (45.71%) during the 3 months post-intervention period, which was significant (p<0.05) in 4(11.4%) surgical units. Further, this effect was sustained in 3 (8.6%) of these units during the 6 month post-intervention period. Significant decrease (p=0.02) in the trend was observed in 1 (2.8%) unit during the entire post-intervention period. Overall reduction of antibiotic consumption (1.88%) was observed, with an increase in the use of low end antibiotics (penicillin, 2nd generation cephalosporins and clindamycin) and decrease in use of high end antibiotic (3rd & 4th generation cephalosporins, β lactam inhibitors, quinolones, aminoglycosides, carbapenems, glycopeptides, linezolid, colistin and tigecycline). This switch may contribute beneficially in reducing overall AMR.

Conclusion: Our study demonstrates a weak impact of FGD in changing antibiotic prescribing behavior. Further analysis of sustainability of FGD and its long term impact on AMR needs to be evaluated. As persuasive interventions rely on behavioral change of individual prescribers, an understanding of the barriers and facilitators of behavior change need be understood first and the interventions need be targeted where desired. Multifaceted interventional efforts are the need of the hour for an effective antimicrobial stewardship programme. The effect of continuous educational sessions cannot be ignored.
Newer trends in microbes and antibiotic sensitivity pattern of community acquired pneumonia in a tertiary care hospital in India
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**Background:** Emergence of unusual bacteria and resistance to antibiotics has made the treatment of community acquired pneumonia an enormous challenge. Study was undertaken to bring light to the recent trends in organisms and antibiotic sensitivity, which would help in tackling the menace of CAP at presentation itself. The study attempts to look into newer trends in the etiology and antibiotic sensitivity pattern in patients hospitalized with community acquired pneumonia in a tertiary care hospital in South India.

**Methods & Materials:** A prospective observational study, of 100 adult patients admitted with a diagnosis of community acquired pneumonia in the medical wards and Intensive care unit of tertiary centre in South India, during a period of 1 year. Data were collected based on detailed patient interview, clinical examination and laboratory investigations. The latter included sputum culture and sensitivity pattern. These were tabulated and percentage incidence of etiological pathogens calculated. The antimicrobial sensitivity pattern was expressed as antibiograms.

**Results:** Klebsiella pneumonia was found to be the most common etiological agent for CAP, in our hospital setting. The other organisms isolated in order of frequency were Streptococcus pneumoniae, Pseudomonas aeruginosa, Escherichia coli and Moraxella catarhalsis. K. pneumoniae was most sensitive to Carbapenams followed by cefoperazone-sulbactum. Sensitivity to other common empirically used antibiotics were less. Presence of ESBL producing klebsiella were detected among the ICU patients. Overall, the common pathogens causing CAP showed highest sensitivity to Carbapenams followed by cefoperazone-sulbactum and piperacillin-tazobactum.

**Conclusion:** In hospital setting, empirical management for cases of CAP should be based on local bacteriological profile. The present study has shown K.pneumoniae as the most common pathogen and cefoperazone-sulbactum as the most likely effective antimicrobial in hospitalized patients with CAP. Possibility of Extended spectrum beta lactamase producing klebsiella should be considered when elderly patients with multiple co-morbidities admitted with CAP in ICU. Indiscriminate use of carbapenams can be avoided in these patients, since cefoperazone sulbactum also caters to the need, leading to better antibiotic stewardship.
Empiric antimicrobial therapy for different types of gall bladder pathologies based on bacterial etiology
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Background: Cholecystectomy is a clean contaminated surgical procedure unless infected bile spills into the peritoneum. A single dose of Cefazolin is recommended for antimicrobial prophylaxis in high risk patients undergoing cholecystectomy. With drug resistance being common in the community, there is need to formulate empiric antibiotic policies based on local etiological data.

Methods & Materials: This was a prospective study conducted from April 2015 to May 2015. Gall bladder swabs, bile & pus samples were accepted as samples and processed for aerobes & for anaerobes using conventional media and special media. Identification of aerobes and anaerobes was performed using Vitek cards. The AST for aerobes was performed on the Vitek. The antibiotic susceptibility testing (AST) for anaerobes was performed using E tests. Patients were classified on the basis of the different gall bladder pathologies based on clinical diagnosis, laboratory parameters like total leukocyte counts, C-Reactive protein and radiological investigations like ultrasound (USG) of the abdomen, Computerised tomography (CT) abdomen, ERCP /MRCP for common bile duct calculi. They were also risk stratified based on previous healthcare contact, antibiotic exposure, diabetes mellitus and prior invasive procedures.

Results: 91 specimens were processed during the study period. 36(42%) were culture positive. 28(73%) culture positives were monomicrobial & 10 (27%) were polymicrobial. E.coli & Enterococcus were the predominant gram negative& gram positive organisms respectively. AST showed 40% ESBLs & 20% Carbapenam Resistant Organisms. History of previous healthcare contact & consumption of antimicrobial agents were important risk factors for acquisition of drug resistance. Beta lactam + Betalactamase (BL/BLI) with or without Aminoglycosides were good empiric options sparing carbapenems. Both gram positive & gram negative organisms showed < 50% susceptibility to Fluoroquinolones.

Conclusion: Cholecystectomy for asymptomatic cholelithiasis in a low risk patient performed laproscopically does not require prophylactic antibiotics. Cefazolin, cefuroxime or Aminoglycosides may be useful as single dose prophylactic agents in low risk patients with other gall bladder pathologies. For high risk patients empiric therapy should cover for Enterococci and aerobic gram negative bacilli. Fluoroquinolones have no role to play in empiric therapy for gall bladder infections.
Missed opportunities for shared decision making in antimicrobial stewardship: The potential consequences of a lack of patient engagement in secondary care

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Background: Within infectious diseases in secondary care, understanding of the potential for behavioural changes arising from patient involvement in antimicrobial decision making is lacking. Shared decision making is becoming part of international policy. The United States have passed it into legislation and the United Kingdom has implemented a number of national interventions across healthcare pathways. This study aims to understand the level of patient involvement in decision making around antimicrobial use in secondary care and the potential consequences associated with it.

Methods & Materials: Fourteen members of the public who had received antimicrobials from secondary care in the preceding 12 months were recruited to participate in group interviews. Group interactions were audio-recorded, transcribed verbatim, and thematically analysed.

Results: Participants reported feelings of disempowerment during episodes of infection in secondary care. Information is currently communicated in a unilateral manner with individuals ‘told’ that they have an infection and will receive an antimicrobial (often unnamed), leading to loss of ownership, frustration, anxiety and ultimately distancing them from participation in decision making. This poor communication drives individuals to seek information from alternative sources, including on-line resources, which are associated with concerns over reliability and individualisation. This failure of communication and information provision from clinicians in secondary care influences individual’s future ideas about infections and their management. This alters their future actions towards infections and antimicrobials and can drive non-adherence to prescribed antimicrobial regimes and loss-to-follow-up after discharge from secondary care.

Conclusion: Current infection management and antimicrobial prescribing practices in secondary care may be failing to engage patients in the decision making process. It is vital that secondary care physicians do not view infection management episodes as discrete events, but as cumulative experiences which have the potential to drive future non-adherence to prescribed antimicrobial regimes and thus poor individual outcomes and antimicrobial resistance. This lesson is transferable to all settings of healthcare, where poor communication and information provision having the potential to influence future health seeking behaviours. We call for the development of clear, pragmatic mechanism to support healthcare professionals and patients engage in infection related decision making during consultations.
Microbial profile of prosthetic joint infections and effectiveness of cefuroxime prophylaxis: Experience from a tertiary care hospital

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**Background:** Prosthetic Joint Infection (PJI) is a serious and devastating complication of total joint arthroplasty (TJR). Currently, second generation cephalosporins (cefuroxime or cefazolin) are the preferred antibiotics for prophylaxis in TJR. The aim of the study was to determine the microbial profile of PJI and assess the effectiveness of cefuroxime as antibiotic prophylaxis.

**Methods & Materials:** Patients with suspicion of PJI as per musculoskeletal infection society (MSIS) criteria were screened from June 2013 to June 2015. Each patient had multiple site samples (pus, synovial fluid & periprosthetic tissues). All samples were cultured aerobically and anaerobically as per standard microbiological practice. Antibiotic susceptibility of the isolates was performed according to Clinical Laboratory Standards Institute (CLSI) guidelines.

**Results:** A total of 54 patients were enrolled of which, 34 were referred from peripheral centers for management of suspected PJI. All the patients received Cefuroxime as antibiotic prophylaxis at the time of both primary and revision arthroplasty. Thirty-six patients were diagnosed to have PJI by microbiological criteria. Gram-negative aerobes were most frequently isolated (64%). Polymicrobial infections were present in 8% of cases. No anaerobes were isolated. The most common isolates were Staphylococcus aureus (23%) followed by Escherichia coli & Pseudomonas aeruginosa (18%) and Klebsiella pneumoniae (15%). Methicillin resistance was noted in 22% of the isolates. Fifty four percentages of gram-negative isolates were Multi Drug Resistant (MDR). In 87% of patients, the microorganisms cultured were not susceptible to cefuroxime. All the gram-negative isolates were uniformly resistant to cefuroxime whereas only 36% of gram-positive isolates were susceptible. Gram-positive isolates were uniformly susceptible to vancomycin, teicoplanin and linezolid; for gram-negative bacilli colistin followed by tigecycline and imipenem showed good activity.

**Conclusion:** Compared to Western literature a predominating MDR gram-negative aetiology of PJIs was noted. Uniform resistance of all the gram-negative isolates to cefuroxime has raised serious concerns about continuing with the practice of using this drug for prophylaxis against PJI at our center. The antibiotic prophylactic regimes should be based on a local knowledge of microbial profile and susceptibility patterns of the causative microorganisms to decrease the incidence of PJI.
Antibiotic prescribing to the inpatients diagnosed with Malaria and Viral fever in two tertiary care hospitals in Madhya Pradesh India  
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**Background:** Indiscriminate antibiotic prescribing is cause for the global increase in antibiotic resistance. Hospitals are major antibiotics users and thus substantially contribute in the development of resistant bacterial strains. The situation is highly under-estimated due to the paucity of studies from major antibiotic consumer countries like India. Aim of the present study was to describe and compare antibiotic prescribing among in-patients diagnosed for non-bacterial infections, at the medicine departments of two private sector hospitals, a teaching (TH) and a non-teaching (NTH), in Madhya Pradesh, India.  

**Methods & Materials:** The data was collected manually for all in-patients for 3 years between 2008 and 2011. Patients were grouped using International Classification of Diseases-10 system for the recorded diagnoses. Patients having bacterial infections were excluded from analysis. Prescribed antibiotics were classified based on WHO anatomical therapeutic chemical (ATC) classification system and Defined daily doses (DDDs) per 1000 patients were calculated. Type and class of prescribed antibiotics and adherence to the generic name prescribing and National list of essential medicines of India (NLEMI) were analyzed.  

**Results:** Overall, 20303 patients were admitted in the medicine departments of two hospitals, of which 66% were prescribed antibiotics. Malaria or viral fever was diagnosed in 693 patients in the TH and 1177 in the NTH. Of these, 82% patients at the TH and 71% at the NTH (71%, p<0.001) were prescribed antibiotics. Prescriptions made at the TH show more adherence both towards the use of generic names and the NLEMI, compared with the NTH (p<0.001). Most commonly prescribed antibiotic classes at the TH were fluoroquinolones (48%) and third generation cephalosporins (21%) and at the NTH were third generation cephalosporins (47%), and fixed dose combinations (19%). The most prescribed antibiotic substances at the TH was ciprofloxacin (1940 DDD/1000 patients), and at the NTH was ceftriaxone (1052 DDD/1000 patients).  

**Conclusion:** Frequent and unnecessary antibiotic prescribing practices at both hospitals were observed. Significantly high percentage of patients in non-bacterial infection groups i.e. malaria and viral fever, were prescribed antibiotics which is a point of concern. An urgent need is felt to develop and implement relevant antibiotic stewardship program to rationalize the antibiotic prescribing in the settings.
Adherence to antiretroviral drug treatment ARV among people living with HIV/AIDS: A study from Eastern Nepal
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Background: HIV/AIDS has threatened an enormous worldwide challenge on the survival of mankind. Antiretroviral therapy (ART) for HIV is increasingly being introduced and utilized in diverse areas of the world. However, little research exists on adherence to ART in different cultural settings, particularly in developing countries such as Nepal. This study aimed to determine adherence to ART and identify associated factors with adherence among people with HIV/AIDS and receiving ART/ARV therapy.

Methods & Materials: In this cross sectional study total of 300 HIV positive subjects were interviewed using semi-structured questionnaire. Study subjects were randomly selected from different HIV clinics of three districts; Sunsari, Morang and Jhapa of Eastern Nepal. Informed & understood written consent was taken and confidentiality was maintained throughout of the study.

Results: The median age for patients was 34 yr. Majority of the respondents were using a non protease inhibitor (PI) treatment regimen (98%). Mean 4-day adherence was 92 %. Adherence was lower over longer periods of recall; Twenty percent reported missed does over the past 7 days; 33 % reported ever missing a full day’s medications and 16 % had a treatment interruption of more than 7-days at least once. On univariate analysis less than university education, being unemployed, obtaining free treatment, severe depression, hospitalization >2 times, having moderate to severe side-effects and taking 4 or more medicines were associated with lower adherence (<90%). However, only obtaining free treatment (adjusted OR, 4.05, 95% CI 1.42-11.54, P=0.009) and severe depression (adjusted OR 4.48, 95% CI 1.64-12.27, P=0.003) were associated with lower adherence in multivariate analysis.

Conclusion: Although the overall adherence was high, lower levels of adherence were documented among poor patients receiving free ARV/ART. Provision of free treatment of ART and side effect management should make available up to unreached poor people of community.
The antimicrobial and phytochemical analysis of the leaves of aspilia africana on clinical isolates

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**Background:** The uses of medicinal plants for treatment of various infections in traditional communities have been an age-long practice. This provides the rationale to study medicinal plant extracts as a possible source of alternative therapy against infections.

**Methods & Materials:** The current study was undertaken to evaluate the phytochemical and antimicrobial properties of *Aspilia africana*. The antimicrobial activity and minimum inhibitory concentration (MIC) of the extracts of *Aspilia Africana* were evaluated against eight organisms - *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida albicans*, *Aspergillus niger*, *Penicillium* spp and *Fusarium* spp. The ethanolic and aqueous extracts were obtained by standard methods. Antimicrobial activity was conducted using a modified agar well diffusion method.

**Results:** The phytochemical screening and analysis carried out in this study showed that the plant extracts contain alkaloids (6.35%), saponins (3.26%), flavonoids (2.01%), tannins (0.88%) and phenols (0.11%). The result showed that ethanolic extract of *Aspilia africana* exerted antimicrobial effect on the test organisms at 25mg/ml, 50mg/ml and 100mg/ml concentrations, while the hot aqueous extract exerted antimicrobial effect at 100mg/ml only on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ethanolic extract of *Aspilia Africana* showed the highest antimicrobial activity with diameter of zone of inhibition of 3.35mm to 17.9mm at 100mg/ concentration. The minimum inhibitory concentration (MIC) of the ethanolic extracts was at a concentration of 25mg/ml.

**Conclusion:** The antimicrobial activity of the extract could be enhanced if the components are purified. This plant therefore holds a promising potential source of new drug for treating infections caused by these clinical pathogens.
Randomized equivalence trial of amoxicillin versus placebo for fast breathing pneumonia (RETAPP)

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**Background:** Fast breathing pneumonia or isolated tachypnea in children, is presumed to be mostly viral in origin. WHO guidelines recommend 3 days of amoxicillin therapy for all children with fast breathing pneumonia in resource limited settings. The recommendations have arisen largely out of hospital studies where the spectrum of disease is more severe. High quality clinical trial evidence to challenge or support the continued use of antibiotics, in low-resource community settings is lacking.

**Methods & Materials:** A community based randomized double blinded placebo-controlled non-inferiority trial is being conducted at primary healthcare centres located in two low income squatter settlements of Karachi, Pakistan. Children aged 2-59 months with WHO defined fast breathing pneumonia are included if they have no danger signs, SaO₂ ≥ 90% on pulse oximetry, absence of use of antibiotics in last 48 hours, no bulging fontanels, pedal edema, asthma, tuberculosis or congenital heart disease. Children are being randomized to receive either 3 days of oral Amoxicillin (standard) or matching placebo (intervention), with 1215 children to be enrolled in each arm. Primary outcome is the difference in treatment failure rates between the two groups, defined as a new clinical sign based on preset definitions indicating illness progression or mortality on day 0, 1, 2 or 3 of therapy.

**Results:** From September 2014 till August 2015, a total of 19,363 children were triaged. Among these, 11,161 (58%) presented with cough or difficulty in breathing. 2,216 (20%) met the inclusion criteria i.e. have history of cough for less than two weeks and have tachypnea. About 40% of all fast breathing under 5 years occurs in babies 2-11 months of age. Of these children with fast breathing, 1056 children have been enrolled so far. The overall treatment failure is 3% and no mortality.

**Conclusion:** The overall rates of treatment failure and relapse in fast breathing pneumonia are low. The trial results will strengthen the evidence to support or refute the use of antibiotics in WHO-IMCI management of pneumonia. Findings will be generalizable to resource limited settings with low HIV and malaria prevalence and Hib and Pneumococcal vaccines in their national immunization plan.
Background: A renewed interest in the usage of polymyxins has been observed, as they are the only treatment option left for multidrug resistant (MDR) and pan-drug resistant (PDR) pathogens like Acinetobacter baumannii. However, knowledge of the pharmacokinetics (PK) and pharmacodynamics (PD) of polymyxins is limited, resulting in inappropriate dosing, potential toxicity and development of resistance. We planned to conduct this prospective PK-PD study of intravenously administered colistin in Indian patients with MDR Gram-negative infections to estimate the levels of both total and free colistin.

Methods & Materials: This was a prospective PK-PD study of intravenously administered colistin in ten patients with MDR Gram-negative infections. The recommended systemic dose of prodrug, colistin methanesulfonate (CMS) was given as 4–6 mg/kg per day as a short-term infusion (for 1 hour) or 1–2 million IU/day in three divided doses (12,500 IU/1mg of CMS). Highly sensitive UHPLC-MS/SRM method was developed and validated to quantify free and total colistin from human sera. Correlation between the predictor variables like AUC/MIC ratio was performed using GraphPadInstat ver. 6 for Mac version 10.10.1 (GraphPad Software, San Diego CA: www.graphpad.com).

Results: The area under the plasma concentration-versus-time curve over 8 hrs (AUC0–8) for free and total colistin ranged after 5th dose from 28.2 to 126 mg*h/liter and 25.8 to 404.9 mg*h/l respectively. All the follow up blood cultures were sterile and majority of the patients survived.

Conclusion: This is the first Indian study where free colistin levels were also estimated. The desired colistin levels to be effective without giving the loading dose were achieved in these Indian patients. This study challenges the pharmacokinetic rationale for a loading dose similar to a study by Gregoire N, et al (2014).
The sensitivity to antibiotics of nosocomial strains of acinetobacter baumanii isolated in the tertiary hospitals in the Central Kazakhstan

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Background: Acinetobacter baumanii are one of main bacterial pathogen caused nosocomial infection according International Guidelines of Infection Control (2015). Last 4 years the part of nosocomial infection caused by Acinetobacter baumanii are dramatically grows.

Methods & Materials: In the multicenter study 200 strains of Acinetobacter baumanii were collected in period 2012-2015yy. Strains were collected in 3 tertiary hospitals in the Central Kazakhstan. All strains were identified by MALDI-TOF mass-spectrometry and typed by PCR detection of OXA-51 carbapenemase as A.baumanii specific label. The sensitivity testing were by micro dilution methods with CLSI criteria using. The OXA-23 and OXA-40 carabapenemases genes detection made by PCR with commercial kits (Interlab Service, Russia). The statistical analysis (MIC90, average MIC, 95% Confidential Interval) was made by WhoNet 6.2 database.

Results: All isolated strains are resistance to main part of antimicrobial drug (pic. 1). During fourth years period the resistance to carabapenems were increased: to imipenem 64,5%; 95%CI 45,5-80,2 (2012 year) to 81,2; 95%CI 66,8-90,5 (2015 year). The resistance growth by logarithmic depence (y = 12,257ln(x) + 65,537; $R^2 = 0,9612$). The testing of general linear hypothesis in regression situation for logarithmic model can predict level of resistance in 2016 at over 85% (pic.2). The dynamic of increasing to meropenem was the same and changed from 61.3% (2012) to 84,5% (2015y). In all cases of resistance to carabapenems the gene blaOXA-23 carbapenemase was detected. The quantiative characteristics of sensitivity to antimicrobials are present in table 1. The high part of studied strains were sensetivity to aminoglycosides: netilmicyn (97,9%), sisomycin (91,3%), tobramycin (100%) and colistine (89,6%) and tigecycline (100%). However all preparations mentioned above are not registered in Kazakhstan so can't using for treatment infections caused A.baumanii.

Table 1. The sensitivity to antimicrobials of nosocomial strains A.baumanii isolated in fourth tertiary hospitals in the Central Kazakhstan (2012-2015yy.)

Conclusion: The resistance to carabapenems in the fourth tertiary hospitals in the Central Kazakhstan are increased during 2012-2015yy. The major cases of resistance to beta-lactams were linked with OXA-23 carbapenemase production. Some part of antibiotics (netilmicyn, sisomycin, tobramycin, colistin and tigecycline) has high activity against studied nosocomial strain of A.baumanii but this drug not registered in Kazakhstan.
Computer assisted rational design and synthesis of some novel 2,4-di-substituted thiazole derivatives and their metal complexes (copper, cobalt, and nickel) as inhibitor of bacterial metabolic enzymes

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**Background:** Recent clinical reports have highlighted the increasing occurrence of methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE) and other antibiotic-resistant human pathogenic microorganisms. Transition metal complexes of heterocyclic moieties or with Schiff's base components have been reported to show promising nucleolytic activity. In an effort to develop newer generation low molecular weight ligands, few thiazole coupled with Schiff's base and their metal complexes were synthesized and biologically evaluated.

**Methods & Materials:** A novel series of thiazole derivatives were rationally designed by Computer Assisted Drug Design (CADD) approach (VLife Science®). The optimized scaffold was synthesized in multiple steps from thiosemicarbazide and was suitably cyclized to get corresponding active 2,4-di-substituted thiazole derivatives. Metal complexes of thiazoles were further prepared using salts of d-block compounds (copper, nickel and cobalt) as per protocol designed in our laboratory. The modern analytical techniques (UV-Vis, IR, NMR, MS, RS, XRD, AAS, MM, MC) revealed that the results were in full agreement with their assigned chemical structures. All the synthesized compounds were screened for their anti-microbial activity according to standard protocol against bacterial strains (E. coli, MTCC-1687; S. aureus, MTCC-2940; B. subtilis, MTCC-441; and K. pneumonia, MTCC-3040). The nucleolytic activity of compounds and metal complexes were evaluated using gel electrophoresis employing E. coli plasmid pBR322.

**Results:** Among them, para-substituted (halogenated) thiazoles exhibited excellent anti-bacterial activity against E. coli, S. aureus, and K. pneumonia. Nearly all compounds exhibited moderate to high nuclease activity. The copper complexes showed moderate nuclease activity while cobalt and nickel complexes displayed excellent nuclease activity. The in silico docking study performed revealed the binding orientations of these compounds at active site amino acid residues ASN 165 and GLU 40 (PDB ID: 1AHP), amino acid residues TYR 151 and GLY 18 (PDB ID: 2C44) and amino acid residues ARG 73 and ASP 76 (PDB ID: 1K6W) of crystal structure of E. coli metabolic enzymes.

**Conclusion:** From this study, it can be concluded that the novel molecules have tremendous anti-microbial and nuclease activity. This research helps in understanding mechanism of action of thiazole based anti-microbials and it may possibly be used as template for searching potent anti-microbial agents in future.
Incidence of SHV and CTX-M Extended spectrum β-lactamases producing gram negative bacterial isolates from antenatal mother with asymptomatic bacteriuria

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Background: Asymptomatic bacteriuria (ABU) occurs in 2 to 10% of cases during pregnancy and the risk of onset of bacteriuria is maximum between 9th to 17th week of gestation. ABU will lead to adverse anomalies if left untreated such as acute pyelonephritis, low birth weight infants (LBW) and premature delivery. The incidence of Extended Spectrum Beta-Lactamases (ESBLs) such as TEM-1, SHV-1 and CTX-M type producing uropathogenic bacteria have been increasing over years. Thus this study was carried out to analyze the population of ESBL producing MDR Gram negative bacteria and to ascertain the most prevalent ESBL gene in our geographical region among antenatal women with asymptomatic bacteriuria.

Methods & Materials: A total of 637 asymptomatic antenatal mothers who consecutively attended the Obstetrics OPD between April and September 2015 were included in this study. Their mid-stream urine samples were collected during the 16th week of gestation and processed in the Department of Clinical Microbiology, MGMCRI following standard methods. Gram negative pathogens were isolated and phenotypically confirmed for ESBL production. All those ESBL positive isolates were screened by PCR for the presence of blaCTX-M, blaSHV and blaTEM with specific primers and the amplicons were subjected to sequencing.

Results: Out of 637 antenatal mothers with asymptomatic bacteriuria, 54 urine samples were not included due to improper sample collection and 268 samples were found to be sterile. Remaining 315 samples showed significant growth of single pathogenic bacteria. Among these, 44 were Gram positive isolates and 271 were Gram negative pathogens. ESBL production was phenotypically observed among 35% (n=95) of these GNB.

Out of these 95 ESBL isolates, 73% were MDR isolates, none of them carried blaTEM and the presence of blaCTX-M and blaSHV were observed in 69.4% and 20% of isolates respectively. All the blaCTX-M and blaSHV amplicons were confirmed through sequencing followed by BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Conclusion: Presence of blaCTX-M and blaSHV genes reflects their prevalence among the community. Periodic and continuous antenatal follow-up only can reduce the complications of asymptomatic bacteriuria among antenatal mothers. This study urges the need for the regional antimicrobial practicing policy by the stakeholders to implement and to monitor.
Molecular detection of azithromycin resistance mechanisms in typhoidal salmonellae

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Background: Antibiotic resistance in enteric fever continues to pose major therapeutic challenge with increase in ciprofloxacin resistance. Azithromycin has been used to treat enteric fever as an alternate treatment option without any guidelines for laboratory interpretation. In 2015 issue CLSI has added breakpoints for testing azithromycin susceptibility in typhoidal Salmonellae. We aimed to assess the prevalence of resistance to azithromycin in S. Typhi and S. Paratyphi A in a collection of strains to characterize the mechanisms underlying resistance.

Methods & Materials: A total number of 224 S. Typhi and S. Paratyphi A isolates were available in the cryopreserved stock which were isolated from the patients presented with enteric fever during 1990 to 2015. Antimicrobial susceptibility testing was done by disk diffusion method as per CLSI 2015 and MICs were determined by E test method as per manufacturer's guidelines (AB Biodisk, Sweden). For molecular characterization of azithromycin resistance, PCR detection was done to screen for the presence of genes responsible for azithromycin resistance i.e, ereA, ereB, ermB, mefA, mphA, mphB and mphD from plasmid and genomic DNA. Sequence analysis was done to detect mutations in acrR, rlpD and rlpV from genomic DNA.

Results: It was observed that 96.11% of the isolates were susceptible to azithromycin using disk diffusion method. There was a linear trend observed with time in azithromycin susceptibility ($\chi^2 = 5.240$, P value <0.02). The MIC 50 and MIC 90 values were 6 and 12 $\mu$g/ml respectively while the resistance breakpoint is $\geq 16$ $\mu$g/ml. There was no acquired gene for macrolide resistance in plasmid or genomic DNA of any isolate and DNA sequences of acrR, rlpD and rlpV genes did not show any mutations.

Total use of azithromycin in pediatric population was only 13,125 mg given to 16 patients in 2014-15.

Conclusion: Azithromycin is a good promising agent against typhoid fever on the basis of MIC distribution in India at present. More studies are required to study the use of azithromycin in complicated infections as at present it is being used only for uncomplicated cases.
In vitro and in vivo activity of “compound A” against gram-positive and -negative pathogens including MDR strains

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Background: Compound A is a novel Topo-IV inhibitor with broad spectrum activity against both Gram-negative and positive pathogens, especially Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, and MRSA. The present study focuses on its in vitro activity, in vivo efficacy and pharmacokinetics-pharmacodynamics in murine infection models.

Methods & Materials: MIC were determined by CLSI method against 135 bacterial strains including ATCC and clinical isolates of (sensitive and MDR) Pseudomonas aeruginosa (n=55), A. baumannii (n=25), Escherichia coli (n=30) and MRSA (n=25) including MDR isolates. Time kill study was performed against these pathogens. The therapeutic efficacy was evaluated using a mouse pulmonary infection model. The pharmacokinetics-pharmacodynamics (PK-PD) parameters against P. aeruginosa were determined in neutropenic murine thigh infection model.

Results: Compound A showed MIC₉₀ of 4, 0.5, 0.25 and 0.25 µg/ml against P. aeruginosa, A. baumannii, E. coli and MRSA, respectively. In murine pulmonary infection model, it showed killing potential against P. aeruginosa and A. baumannii and reduced the pulmonary bacterial number in a dose-dependent manner. In the PK-PD study, the efficacy of Compound A against P. aeruginosa infection was correlated with AUC/MIC (R² = 0.89) and Time above MIC (R² = 0.93) more than Cmax/MIC (R² = 0.12).

Conclusion: Compound A exhibited excellent broad spectrum of activity against Gram-positive and negative pathogens. Hence, it could be a promising investigational candidate for antibacterial therapy.
A small molecule that inhibits FtsZ with potent in vitro and in vivo activity against staphylococcus aureus

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**Background:** The emergence of bacterial resistance to antibiotics is a major concern; therefore, it is critical to develop new antibiotics with novel modes of action. FtsZ is an essential bacterial guanosine triphosphatase (GTPase) play an essential role in bacterial cell division, and its homologs are present in almost all eubacteria, archaea and mammalian that polymerizes and assembles into a ring to initiate cell division. Compound-A is a novel FtsZ inhibitor with potent and selective *in vitro* bactericidal activity against methicillin-susceptible *Staphylococcus aureus* (MSSA) methicillin-resistant *Staphylococcus aureus* (MRSA).

**Methods & Materials:** The antibacterial activities were determined by the CLSI micro-broth dilution methods. *In vivo* efficacy was evaluated in mouse model of infection caused by MRSA.

**Results:** Compound-A showed potent activity against various clinical isolates of MSSA and MRSA. The MIC⁹⁰s of Compound-A against MRSA was 2 µg/mL, respectively. Compound-A showed the potent inhibitory activity against *S. aureus* FtsZ enzyme >100 fold selection against its mammalian homolog porcin β-tubulin. Compound-A showed bactericidal activity against MRSA and showed synergy with carbapenem drugs. Compound-A was efficacious in mouse infection model.

**Conclusion:** Compound-A showed potent activity against multidrug resistant MRSA strains and bactericidal activity. These results suggest that Compound-A is suitable for further investigation.
BBIL-5: An investigational new biotherapeutic for treating drug resistant S.aureus infections

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Background: With development of resistance to new generation antibiotics, very few therapeutic options are left in the arsenal against Staphylococcus aureus. Virulence factors and resistant traits emerging in the hospital and in community acquired infections favor colonization and pathogenesis. BBIL-5 is a potent anti-Staphylococcal protein that kills S.aureus of any antibiotic resistance profile.

Methods & Materials: Minimum inhibitory concentration (MIC) of S.aureus clinical isolates to BBIL-5 was estimated by broth dilution method. Antibiotic susceptibility was determined by Stoke’s method and by disk diffusion studies. Synergy with antibiotics was demonstrated by kill kinetics and in vivo animal studies. Therapeutic dose regimen was established by infection with intraperitoneally administered 10⁷-10⁸ S.aureus, and subsequent treatment with BBIL-5 (doses in mg/Kg body weight). Pharmacokinetic/Pharmacodynamics (PK/PD) indices were determined for single and multiple doses administered intravenously in mice and rabbits. BBIL-5 concentration in PK studies and antibodies to the protein were estimated by ELISA. Recombinant production of BBIL-5 in E.coli and product development has been advanced to GMP. GLP pre-clinical toxicity studies are in progress.

Results: The MIC to BBIL-5 ranged from 2-32 ng/ml. No antibiotic cross resistance was observed. Standardized doses of BBIL-5 when administered intravenously in three bolus doses, q24h completely eradicated S.aureus infection in mice. A single therapeutic dose of the GMP product provided 2–3 Log reductions in colony forming units (CFU) of S.aureus. Synergy with beta lactams reduced the therapeutic dose to one-fifth of the standardized dose. AUC/MIC and Cmax/MIC were the pre-dominant PK/PD indices. A high ratio of Cmax/AUC and AUC/MIC positively improved the outcome in eradication of S.aureus. Pharmacokinetic profile in animals showed first order kinetics of elimination where C vs. t graph is not linear, but is a decaying exponential, while Log C vs. t graph is linear. Improved therapeutic efficacy against systemic S.aureus infections was achieved by reducing kel (elimination rate constant) by IV infusion. The protein was minimally immunogenic due to short circulating half-life. The GMP product was extensively characterized.

Conclusion: BBIL-5 is the first investigational biotherapeutic of its kind with demonstrated efficacy in treating multi-drug resistant S.aureus infections.
In-vivo efficacy of a novel Leu-t-RNA synthetase inhibitor compound a against MDR Pseudomonas aeruginosa 1594965 in a foreign body associated urinary tract infection model

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**Background:** *Pseudomonas aeruginosa* is one of the major pathogens causing morbidity and mortality in hospital acquired infections (HAI). MDR *P. aeruginosa* are resistant to 3 or more classes of antibiotics. In cUTI cases catheter associated infections are incurable by most of the antibiotics. Compound A a novel Leu-t-RNA synthetase inhibitor showed efficacy in mouse ascending urinary tract infection (AUTI) model against *P. aeruginosa* biofilms formed on urinary catheters.

**Methods & Materials:** In this study we established a mouse model of foreign body associated AUTI. A spiral polyethylene tube (PT) was placed transurethrally into bladder without surgical manipulation, followed by transurethral inoculation with *Pseudomonas aeruginosa* 1594965 (MDR) strain. Scanning electron microscopy (SEM) was done after 4h of inoculation of bacteria and bacterial counts were taken in kidneys, bladders and catheter pieces for respective samples. *In vivo* efficacy of Compound A was tested using this established model against *P. aeruginosa* 1594965 (Compound A MIC: 1 µg/ml, meropenem MIC: 32 µg/ml). Compound A was tested at 30 and 220mg/kg SC q6h and meropenem was tested at human simulated dose of 60 mg/kg SC q6h. The treatment was initiated 4h post infection and continued daily for 7 days. Bacterial counts of placebo 4h, 7 days and 7 days treated group’s samples of kidneys, bladders and catheters were calculated. Data was analysed using bacterial counts of each group and compared with those of 4 h placebo control group.

**Results:** SEM showed good establishment of biofilm in 4 h samples. In foreign body associated mouse infection model. LRS inhibitor Compound A at 30, 220 mg/kg SC q6h showed log₁₀ difference of -2.38, -2.18 log₁₀ CFU/kidney respectively compared to 4h placebo and meropenem at 60 mg/kg SC q6h was inefficacious. In bladders this log difference was -2.30, -1.21, -0.78 respectively. On catheter only Compound A at 220mg/kg SC q6h showed log₁₀ difference of -1.59 log₁₀CFU/ml. No efficacy was observed in remaining groups.

**Conclusion:** Efficacy of Compound A in foreign body associated mouse AUTI infection model against MDR *Pseudomonas aeruginosa* 1594965 proves its efficacy on biofilm. These results warrant its further investigation in HAI associated *Pseudomonas aeruginosa* infections.
Activity of a novel ketolide A against haemophilus influenzae using in vitro and in vivo pharmacodynamic models

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Background: Haemophilus influenzae causes community-acquired respiratory tract and invasive infections in humans. Resistance to macrolides and fluoroquinolones is emerging in H. influenzae limiting its therapeutic options. In the present study we demonstrated in vitro and in vivo potential of this novel ketolide against H. influenzae.

Methods & Materials: MIC of fresh clinical isolates of H. influenzae (n=145) (β- lactamase producer , non-producer strains and standard ATCC quality control strains) from tertiary care centers in India was evaluated using microbroth dilution method (CLSI). Bactericidal potential was evaluated using time kill kinetics method against 3 strains. Immunocompromised mouse and rat pneumonia model were performed against 2 clinical isolates of H. influenzae. Plasma and ELF concentrations were estimated using standard HPLC analysis and Microbiological method.

Results: Ketolide A showed MIC range of 0.03 - 4 µg/ml against fresh clinical isolates of H. influenzae. Ketolide A was bactericidal against 3 different strains of H. influenzae at 4X MIC concentration and the results were comparable with telithromycin. Ketolide A showed >1 log₁₀ reduction in the CFU/lungs compared to 2 h control at 100 mg/kg BW PO bid in rat and mouse immunocompromised pulmonary infection models. The efficacy of ketolide A correlated with its accumulation in rat lung tissue much above its MIC levels (1-2 µg/ml) upto 8h.

Conclusion: Efficacy of ketolide A in rodent H. influenzae models and high concentration in ELF and lung tissue warrants its further investigation for the treatment of H. influenzae infections.
Synthesis and antibacterial activity of novel 3'-N-alkyl ketolide and fluoro-ketolide carbamates against community acquired respiratory pathogens

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**Background:** Macrolides used in clinic are associated with number of shortcomings. Our research efforts were directed towards identification of novel macrolides that will address these issues.

**Methods & Materials:** We report herein, modifications at 3'-N and C-2 position of the macrolide ring system. A series of ketolides and 2-fluoro-ketolides 11, 12-cyclic carbamates in which one of the methyl of the 3'-N,N-dimethyl group was replaced by the other alkyl group were synthesized and evaluated against relevant macrolide-sensitive and macrolide resistant respiratory pathogens.

**Results:** Excellent in-vitro activities with MIC range of 0.008- 0.125 against gram positive *S.pneumoniae* and 0.06 - 16µg/ml against macrolide resistant *S.pneumoniae* strains were demonstrated for some of the C2-fluoroketolides. Few of them also showed excellent activity against *S. aureus* and *S. pyogenes* strains. Modulation of the activity was also observed with modification on 3'-N position.

**Conclusion:** We have synthesized novel series of ketolide and C2-fluoroketolide antibiotics that exhibited good in-vitro activity against the macrolide resistant pathogens including lab generated telithromycin resistant *S.pneumoniae* strains. Moreover, the structure activity relationship study of these compounds led to the discovery of a novel fluoroketolide lead.
Pharmacokinetic endeavors for antimalarial therapeutics

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Background: The pharmacokinetic compatibility of short-acting CDRI candidate antimalarial trioxane derivative, 99-411, was tested with long-acting prescription antimalarials, lumefantrine and piperaquine.

Methods & Materials: LC-ESI-MS/MS methods were validated for simultaneous bioanalysis of lumefantrine and 99-411 and of piperaquine and 99-411 combinations. The interaction studies were performed in rats using these validated methods.

Results: The total systemic exposure of 99-411 increased when administered with either lumefantrine or piperaquine. However, co-administration of 99-411 significantly decreased the systemic exposure of piperaquine by half-fold while it had no effect on the kinetics of lumefantrine. 99-411, thus, seemed to be a good alternative to artemisinin derivatives for combination treatment with lumefantrine. To explore the reason for increased plasma levels of 99-411, an in situ permeability study was performed by co-perfusing lumefantrine and 99-411. In presence of lumefantrine, the absorption of 99-411 was significantly increased by 1.37 times than when given alone.

Conclusion: Short-acting CDRI candidate antimalarial trioxane derivative, 99-411, was found to be pharmacokinetically compatible with long-acting prescription antimalarials, lumefantrine.
Designing new antimalarial hits from African medicinal plants at the University of Buea (Cameroon); Part I: Isolation, in vitro activity, in silico “drug-likeness” and Pharmacokinetic profiles

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Background: Drug resistance has drastically exacerbated the burden of malaria in Africa. It is therefore an urgent need to design novel therapies both efficacious, safe and affordable especially to poor people of endemic remote areas. The Malaria Drug discovery programme of the University of Buea (Cameroon) aims to identify the compounds responsible for the anti-malarial activity of medicinal plants commonly used in handling malaria symptoms by traditional healers of Cameroon. The present paper report on the potential of selected compounds identified from Dacryodes edulis (Burseraceae), Kigelia africana (Bignoniaceae) and Hypericum lanceolatum (Hypericaceae), and their suitability as leads for the treatment of drug resistant malaria.

Methods & Materials: 17 compounds were isolated from the various extracts of the three plants and tested against both chloroquine-susceptible (3D7, and D6) and multidrug-resistant Dd2, W2, K1 and W2mef) strains of Plasmodium falciparum, using the parasite lactate dehydrogenase method. Cytotoxicity studies were carried out on LLC-MK2 monkey kidney epithelial cell-line. In silico analysis was conducted by calculating molecular descriptors using the MOE software running on a Linux workstation. The “drug-likeness” of the isolated compounds was assessed using Lipinski criteria, from computed molecular properties of the geometry optimized structures. Computed descriptors often used to predict absorption, distribution, metabolism, elimination and toxicity (ADMET) were used to assess the pharmacokinetic profiles of the isolated compounds.

Results: Antiplasmodial activity was demonstrated for the first time in 7 major natural products previously identified in D. edulis, H. lanceolatum and Kigelia africana, but not tested against malaria parasites. The most active compound identified was termed DES4 from D. edulis. with IC50 of 0.37 and 0.55 µg/mL, against 3D7 and Dd2 respectively. In addition, this compound was shown to act in synergy with quinine, satisfied all criteria of “Drug-likeness” and showed considerable probability of providing an antimalarial lead. The remaining four compounds also showed antiplasmodial activity, but were less effective than DES4. None of the tested compounds was cytotoxicity against LLC-MK2 cells, suggesting their selective activities on malaria parasites.

Conclusion: Based on the high in vitro activity, low toxicity and predicted “Drug-likeness” DES4 merits further investigation as a possible drug lead for the treatment of malaria.
Distribution of emm types of beta hemolytic streptococci associated with necrotizing fascitis: Clinical profile and outcome

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**Background:** Necrotizing fasciitis (NF) is a rapidly progressive, potentially life threatening infection with a median mortality rate of 32% which may reach 100% without prompt treatment. Based on etiology necrotizing fasciitis is classified into, type I (polymicrobial infections), type II (monomicrobial infections, classically caused by *Streptococcus pyogenes*), and type III (Clostridial infections). The present study was undertaken to find the prevalence and emm types of beta hemolytic streptococci (BHS) causing necrotizing fascitis and to investigate the clinical characteristics and outcomes associated with it.

**Methods & Materials:** All BHS isolated from necrotizing fascitis cases over a period of two years (1st October 2013 to 30th September 2015) in the Department of Microbiology, JIPMER were included in the study. These isolates were further characterized by bacitracin sensitivity, PYR test, Lancefield antigen detection and spy1258 PCR. PCR amplification and sequencing of emm genes and assignment of emm types was performed as described by the Center for Disease Control and Prevention, Atlanta.

**Results:** Out of a total of 651 cases of NF, 23(3.5%) were associated with BHS. Eight (34.7%) were monomicrobial, and 15(65.2%) were polymicrobial. In monomicrobial NF, the major pathogen was GAS. Among BHS isolates 19 were group A streptococci (GAS), 2 each belonged to group F and group C and 1 was group G. All the 19 M typed isolates (GAS-17, GCS-2, GGS-1) belonged to different emm types (*emm*44 (n=3), *emm*222,2, *emm*4.5, *emm*8, *emm*193, *emm*63, *emm*86.2, *emm* 80, *emm*74, *emm*15.2, *emm*82.1, *emm*113, *emm*110, *emm*209, StC28k, StC1741, StG11).

Of the study population, 18 (78.2%) were men, with a mean age of 54 years. The commonest co-morbidity was diabetes mellitus, followed by chronic kidney disease and alcoholism. The most frequently affected site was the lower extremities. All the patients underwent surgical debridement and amputation was performed in one patient, followed by antibiotic therapy. The survival rate was 100%.

**Conclusion:** The emm gene profile of our study population was entirely different from the common emm types (*emm*1, *emm*3, *emm*28, *emm*18) related to severe disease. The 100% survival rate may be attributed to early appropriate management as well as less virulent emm types.
Background: On the 11th of September 2015, the Borno state ministry of health was alerted by NGOs about 30 cases of acute watery diarrhoea and 7 deaths from two IDP camps in Maiduguri. 6 NFELTP residents were recruited to investigate the outbreak. We decide to conduct a study to identify associated risk factors and identify ways to avert further outbreaks.

Methods & Materials: We conducted a retrospective 1:2 unmatched case control study in order to identify the risk factors associated with the outbreak using a semi structured questionnaire. A case was defined as a person greater than 2 years of age with acute diarrhea with or without vomiting from September 10th to September 21st 2015. We searched for more cases within other camps and went through hospital records. Fecal, water and environmental samples were taken for laboratory analysis.

Results: A total of 385 cases with 13 deaths, in a population of 11,384 was identified. Attack rate: 3.4%, CFR: 3.4% vomiting (44%), fever (31%), abdominal pain (16%), mean age was 31 years (ranges 2-78 years). Vibrio cholera type 01 was isolated only from the fecal sample. Poor hygienic practices sequel to contact with a case OR-2.8, CI (2.1-3.7) was significantly associated with this outbreak. The epidemic curve shows a point source propagated transmission.

Conclusion: Having contact with cases followed by poor personal hygiene was responsible for the propagation and transmission of the outbreak. The index case was infected from outside the camp and subsequent cases were contacts linked to the index case. Knowledge and awareness campaign on preventive measures and improvement of sanitation and personal hygiene was an effective measure in curtailing the outbreak.
A spate of Lemierre syndrome cases: Causes other than Fusobacterium spp.

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Background: Lemierre’s syndrome is a rare condition typified by septic thrombophlebitis of the internal jugular vein (IJV) originating from a pharyngeal septic focus caused by Fusobacterium necrophorum that usually occurs in young adults. Rarer extra-pharyngeal sources and non-Fusobacterial bacteria causing the syndrome have been reported.

Methods & Materials: We describe a consecutive series of 3 rare cases of non-fusobacterial Lemierre’s syndrome of both pharyngeal and extra-pharyngeal origin over a 3-month period in 2014. The first case is a 53-year old Malay female presenting with diabetic ketoacidosis after a recent dental procedure. CT scan showed a left tonsilo-pharyngeal abscess complicated by left external & internal jugular veins thrombophlebitis and septic emboli in both lungs. All cultures including blood, pus from derided abscess and endotracheal aspirates isolated Klebsiella pneumoniae. A subsequent case also involved a 53-year old Malay female with Down’s syndrome presenting with chronic right ear ache. CT showed right malignant otitis externa, and a parotid and peritonsillar abscess complicated by right IJV thrombosis extending to right sigmoid and transverse sinuses. Admitting blood cultures isolated Trueperella bernardiae, Bacteroides stercoris, and Campylobacter urealyticus. The last case is a 51-year old Malay male with a recent history of diffuse large B cell lymphoma treated 6 months prior. He self-extracted a decayed tooth 4 days before symptom onset. CT imaging revealed a left masticator space abscess, with bilateral IJV thrombosis, left cavernous sinus thrombosis, pulmonic emboli and focal cerebritis. Blood culture isolated Streptococcus constellatus and pus from aspirated abscess grew Prevotella buccae.

Results: Despite very ill initial presentations, all our three cases survived after debridement of abscesses and prolonged antibiotic therapy. The latter 2 cases also received anticoagulation therapy. Our patients are unusual due to the older (middle-) age presentation, atypical causative organisms and originating sources.

Conclusion: Clinicians should recognise this rare condition that may be increasing in incidence and ensuing serious complications including septic shock and metastatic disease that may involve the central nervous system.
Analysis of IL-10 and IL-6 gene polymorphisms and their serum levels in patients with brucellosis: A case control study
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Background: It seems that polymorphism in the regulatory areas of cytokine genes affect the cytokine production capacity and may play a role in the development of infectious diseases. Interleukin-10 (IL-10) and Interleukin-6 (IL-6), which are cytokines of Th2 causes the macrophage become inactive and patient conditions get worse.

Methods & Materials: In this case-control study, 60 patients with brucellosis and 60 healthy participants were recruited. IL-10 genotyping at positions -1082 (G/A), -819 (C/T) and -592 (C/A) and IL-6 genotyping at position -174 (G/C) were analysed by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) and restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) methods. The levels of IL-10 and IL-6 were determined by a sandwich enzyme-linked immunosorbent assay in sera of study population.

Results: The AA and CC genotypes of the IL-10 gene at positions -1082 G/A and -819 C/T were significantly more frequent in patients in comparison to controls, respectively. The AG genotype of the IL-10 gene at positions -1082 G/A was significantly more frequent in controls groups than the patients. Serum levels of IL-10 and IL-6 were significantly more frequent in the patients than in the control groups.

Conclusion: Our study showed that the AA and CC genotypes at positions -1082 and -819 are very important, respectively. These results suggest that IL-10 (-1082 G/A) GG genotype may be considered as a risk factor for brucellosis, while the AG genotype might be a protective factor against the disease.
Transcriptome analysis of Salmonella enterica subspecies enterica serotype Typhi Biofilm

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Background: Chronic infection with Salmonella enterica subspecies enterica serotype Typhi (S. Typhi), is associated with long-term localization of the bacteria in the gallbladder in the form of biofilm on the surface of human cholesterol gallstones that can lead to the development of the typhoid carrier state. Therefore, understanding the regulation of biofilm is important to fully comprehend the pathogenesis of S. Typhi and development of the typhoid carrier state.

Methods & Materials: We have successfully standardized a culture method for S. Typhi biofilm and confirmed the morphology by Scanning Electron Microscope (SEM). The good and intact yield of total RNA from biofilm was also standardized by comparing with different extraction methods. The total transcriptome from the exponential phase of the bacteria growth in normal culture medium (planktonic cells), biofilm medium (Intermediate cells), and mature biofilm, were sequenced by next generation sequencing using Illumina Hi-Seq 2000 platform.

Results: Seventy-seven mRNAs were found to be significantly differentially expressed in biofilm cells compare to the planktonic cells with the criteria Padj < 1e-10 and log2 fold change ≥ 2. As expected biofilm-modulation protein, bdm was up-regulated in the biofilm, along with stationary-phase proteins and proteins associated with high osmolarity. An iron transporter component was found to be up-regulated in the biofilm as expected due to the lower nutrient conditions of biofilm formation.

The possible novel non-coding RNA (ncRNA) transcripts were identified using de-novo assembly of all reads with 100-300nt in length. They were investigated for their overlap with non-coding regions, and for differential expression between the 3 conditions. Among them 134 transcripts in biofilm and 36 transcripts in intermediate cells showed significant differential expression compare to planktonic cells. One of the most interesting is the ncRNA transcript_5176, which was down-regulated 14 times in biofilm, while the overlapping messenger RNA (mRNA) was up-regulated 54 times. Interestingly, the up-regulated mRNA was a multiple stress-resistance protein, BhsA and it is involved in biofilm formation.

Conclusion: In conclusion, this study revealed differential expression of mRNAs and ncRNAs during the planktonic, intermediate and biofilm cell transition and thus can help us to understand the gene regulation during biofilm formation of S. Typhi.
Aerobic bacteria in infected breast of Turkish woman: Prevalence and antimicrobial resistance evaluated in cases with lactational mastitis, periductal mastitis and granulomatous mastitis


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**Background:** Mastitis is a disease causing serious psychological and physical difficulties in women. We report the aerobic bacteria isolated from infected breasts of 44 women diagnosed with lactational mastitis (LM), 10 with periductal mastitis (PM) and 46 diagnosed with granulomatous mastitis (GM).

**Methods & Materials:** Between November 2015 - June 2015, pus specimens of 100 women diagnosed with mastitis were Gram stained and aerobic cultures were performed. The isolated bacteria were identified by standard microbiological methods and by Phoenix microbiology analyzer (BD Phoenix™). From 1 LM patient and 19 GM patient’s pus samples which showed bacteria on their Gram-stained smears but had sterile cultures, bacterial DNA was isolated. After extraction, the DNA was purified and PCR amplification procedures were performed. 1446–1515nt amplicons were sequenced using the BigDye Terminator v3.1 kit (Applied Biosystems, USA). DNA sequences were examined and edited using MEGA software. Antimicrobial sensitivities of bacteria isolated from cultures were determined by the disc diffusion method. The results were evaluated according to EUCAST 2015 documents.

**Results:** Aerobic bacteria were isolated from 31(70.4%) LM, 6(60%) PM and 19(41.3%) GM patient’s samples, *S. aureus* was found as the most prevalent bacteria (43.1%, 60% respectively) in LM and PM patients. However *C. kroppenstedtii* was found as the most prevalent bacteria (15.2%) in GM patient’s samples. Furthermore, 2 *S. aureus*, 3 *S. epidermidis*, 1 *S. capitis*, 1 *Streptococcus* spp, 1 *S. mitis*, 1 *L. lactis*, 1 *K. pneumoniae*, 3 *C. kroppenstedtii*, 1 *C. pseudotuberculosis*, 1 *C. urealyticum* DNA were determined from 19(28%) GM patient samples. A statistically significant relation was found between Gram positive bacteria and GM (p<0.05).The most effective antibiotics were trimethoprim-sulfamethoxazole, gentamicin, ciprofloxacin, vancomycin, linezolid for Gram(+)diplococci, ceftazidime, amoxicillin/clavulanic acid, imipenem and gentamicin for Gram(-)bacilli and penicillin, gentamicin, vancomycin, linezolid for Gram(+)bacilli.

**Conclusion:** As a result, for the first time, the bacteria that until now appeared only in animal mastitis (*S. saprophyticus, S. capitis, L. lactis*) were determined on human granulomatous mastitis. We believe that, the addition of antibiotics penetrating strongly to the adipose tissue and effective on the most common isolated bacteria would be beneficial for the treatment of patients with mastitis.
Role of bacteria in Inflammatory bowel disease (IBD)

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Background: Inflammatory bowel disease (IBD) is an idiopathic disease caused by a dysregulated immune response to host intestinal micro-flora. The healthy human intestine is represented by the presence of bacterial communities predominantly belonging to obligate anaerobes; however dysbiosis and dys-anaerobiosis in intestinal micro-flora may lead to the development of inflammatory bowel disease (IBD). Various microorganisms including Clostridium difficile have been implicated in the exacerbation of Inflammatory bowel disease.

Methods & Materials: In this study, gut microbiota of twenty two patients suffering from IBD (acute stage) was compared with gut microbiota of the follow-up samples from same patient (remission stage). Biopsy samples were collected via colonoscopy, and tissue samples were processed immediately upon collection for isolation of DNA. Mucosal microbiota was analysed by means of 16S rRNA gene-based short gun clone library sequencing. Clostridium difficile co-infection during IBD was analyzed using qPCR method.

Results: Analysis of 6437 good quality sequences demonstrated a significant reduction of bacterial diversity consistently from phylum to species level (p-value < 0.05) of individuals in the acute phase of IBD, Significant increase in abundance of unusual aerobes and facultative anaerobes, including members from the phylum Proteobacteria (p-value=0.031) was also observed. Infectious bacterial communities belonging to genus Stenotrophomonas, Parabacteroides, Elizabethkingia, Pseudomonas, Micrococcus, Ochrobactrum and Achromobacter were found to be dominant in the intestine during the acute phase of IBD as compared to IBD patients in remission phase. However our qPCR results suggested no significant correlation between C.difficile bacterial infection and IBD in any stage of the disease.

Conclusion: The reduction of bacterial diversity with an increase in the infectious bacterial communities signifies dysbiosis at mucosal level in patient suffering from IBD, and any single bacterial species like C.difficile may not be solely responsible for exacerbation of IBD.
The gene expression of helicobacter pylori neutrophil-activating protein (HP-NAP), an immunomodulator in allergic asthma: The first case – control study conducted in children living in Istanbul-Turkey

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Background: It was reported that there is an inverse relationship between the presence of H.pylori and asthma and Th2 response may be directed to the Th1 response in asthmatic persons by HP-NAP A. We reporte the H.pylori quantity in the gut microbiota of children with allergic asthma comparing with that of healthy controls. Additionnaly we report the gene expression levels of HP-NAP A.

Methods & Materials: From March 2014 to January 2015 bacterial DNA and RNA were isolated from stool samples of 92 asthmatic children aged from 3 to 8 years and from stool samples of 88 age, and gender matched healthy controls. The quantity of H.pylori was determined by Real Time PCR and cDNA synthesis was made from the isolated RNA. The gene expression studies were conducted with HP-NAP A gene(Accession No: U16121.1) and with 16S rRNA gene, primers, probes, cDNAs and Lightcycler 480 Probe master kit, RD in LightCycler 96 instrument. The Cq values obtained from HP-NAP A gene were compared with that of 16S rRNA reference gene and gene expression analyses were performed using delta delta-Ct(ddCt) method.

Results: H.pylori DNA was found negative in stool samples of all 92 asthmatic children and positive in 18(20,4%) of 88 healthy controls. A statistically significant difference was found between these groups(p< 0,0001, OR =0,79). The quantity of H.pylori determined by qPCR was higher in 7 of 18 H.pylori positive samples but despite that, in 4 of these 7 samples, the detected HP-NAP A expression levels was low comparing with the levels obtained from 8(8/11) of the remaining 11 H.pylori positive samples.

Conclusion: Our findings supports the opinion about the presence of an inverse relationship between H.pylori infection and asthma. Additionnaly, we belive that as well as the presence of HP-NAP A, its expression level plays an important role in the immunomodulation and we can think that its protective effect against asthma is related with the expression level of HP-NAP A rather then the quantity of H.pylori in the fecal microbiota. We belive that more extensive researches with different approaches and different perspectives are needed.
A case of Actinomyces meyeri empyema: still a challenging entity management

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Background: Actinomycosis is an uncommon chronic infection caused by a group of anaerobic Gram-positive bacilli belonging to the Actinomyces genus, being Actinomyces israelii the most frequently implicated in human diseases. Actinomyces meyeri, in dissimilarity to other species of Actinomyces, is an unusual cause of human actinomycosis, regularly causes pulmonary disease and shows a propensity for hematogenous dissemination. Infection of the respiratory system due to A. meyeri presents with nonspecific symptoms and has no distinctive findings of diagnostic value in radiological tests. Consequently, microbiological and histological and examinations are imperative for diagnosis.

Methods & Materials: Here, we report a rare case of empyema caused by A. meyeri.

Results: A 44-year-old male presented with a history of 4 months of dyspnea and chest pain. A large amount of loculated pleural effusion was present on the right side and was documented on radiological studies (Figure 1). A chest tube was inserted and purulent pleural fluid was drained. A. meyeri was isolated in anaerobic cultures of the pleural fluid. The infection was significantly improved in response to treatment with intravenous clindamycin (4800mg daily) and oral clindamicin (450 mg every 6 hours) for a period of 4 months (Figure 2).

Conclusion: A review of the English-language literature revealed only six case reports of A. meyeri empyema, being the present case the first one reported in Portugal. Four of six patients underwent a surgical procedure, and the duration of antibiotic therapy ranged from 4 to 12 months. In comparison to most of the previous reports, the present case was diagnosed early and was effectively drained with only a chest tube. Additionally, there was no evidence of dissemination and symptoms and radiological findings were rapidly improved, demonstrating that short-term antibiotic treatment may be attempted when the adequate management is promptly instituted according to an early diagnosis and if there is no evidence of dissemination. In conclusion, empyema due to A. meyeri is uncommon, and anaerobic culture of pleural fluid plays a main part in the early diagnosis of actinomycosis involving pleura. Although the loculated pleural effusion was large, early diagnosis and successful drainage may abbreviate the duration of treatment.
Detection of (hld) gene from staphylococcus epidermidis strains isolated from ICU of Rasul-e Akram hospital, Tehran-Iran
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Background: Coagulase negative Staphylococci are the most important hospital pathogens. According to the bacterial virulence factors such as potential ability for biofilm formation and also the emergence of methicillin-resistant strains, delta toxin may lead to the great clinical significant concerns. Delta toxin is encoded by the (hld) gene and a similar system called (agr), which is responsible for regulating.

Methods & Materials: In this study, a total of 55 isolates of invasive Staphylococcus epidermidis were collected from different ICU samples of Rasul-e Akram hospital, Tehran, Iran, due to CDC criteria for coagulase negative staphylococci guidelines. All of the isolates were confirmed by API and delta toxin synergistic hemolysis test, finally the prevalence of (hld) gene was estimated by PCR molecular test with specific primers which were designed by primer designer software

Results: Amongst recovered specimens, both blood samples and ear wound infections with (34.5%) and (3.5%) showed the highest and lowest percentage respectively. The synergistic hemolysis was evaluated (58.2%) by phenotypic method, while in genotypic method the frequency of hld gene was determined (74.5%).

Conclusion: The prevalence of delta toxin as an important virulence factor in S. epidermidis is considered as an essential aspect for determination of invasive strains. In similar studies the mentioned factor has been investigated in NICU while in present study we have compared the both NICU and ICU wards.
Single-domain antibody selected from the phage display library neutralizes Escherichia coli endotoxin-induced effects on leukocytes in vitro and in Swiss albino mice

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**Background:** Lipopolysaccharide (LPS), also called endotoxin, released from G−ve bacteria has a predominant role in sepsis through excessive production of pro-inflammatory mediators. Attempts to design rational therapies against endotoxemia and sepsis continue. The objective of the present study was to assess the ability of single-domain antibody clones selected from phage display library to neutralize LPS-induced effects on murine and buffalo leukocytes in vitro and in Swiss albino mice.

**Methods & Materials:** Three dAb.HA clones (Cl-18, Cl-23 and Cl-26) originally selected as LPS-binders from the phage display library of LPS-immunized Indian desert camel were sub-cloned in pET303/CT vector/BL21(DE3) host system and expressed as dAb.6xHis clones. The clones were purified by Nickel-chelate chromatography, and confirmed by SDS-PAGE and immunoblotting. The nucleotide sequences of the clones were determined.

**Results:** All the dAb clones reacted with both LPS and lipid A in indirect ELISA and exhibited thermo-stability. The affinity constants (Ka) of dAbCl-26, dAbCl-18 and dAbCl-23 for LPS were 4.28 x10⁸/M, 2.18 x10⁸/M and 2.19x10⁸/M, respectively. Both dAbCl-26 and dAbCl-18 decreased the LPS-induced expression of TNFα, IL-1β and MHC II genes in buffalo leukocytes, and IL-1β, IL-6, CD80, MHC-II and TNFα (only Cl-26) genes in murine macrophages, but dAbCl-23 increased buffalo TNFα and MHC-II, and the murine genes as measured by RT-qPCR. The dAbCl-26 and dAbCl-18 decreased, but dAbCl-23 increased LPS-induced TNFα levels in Swiss albino mouse model.

**Conclusion:** In conclusion, dAbCl-26 was able to neutralize LPS-induced effects in murine and buffalo macrophages, and in vivo in mice.
Study on the frequency of spa gene in Staphylococcus aureus isolates from human infections and its relationship with mecA gene

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Background: Staphylococcus aureus is the most important cause of nosocomial infections acquired in the community. Today, S. aureus has been known as one of the most important global problems because of its high virulence and increasing resistance to antimicrobial drugs. Due to the high mortality rate of nosocomial infections associated with methicillin-resistant S. aureus, identification and knowledge of the regional model is necessary for the proper treatment of infections caused by this organism. On the other hand, genotyping of the isolates of this bacterium can be widely used because we can identify the source of infection. The molecular techniques have been developed to determine the genetic of the isolates. One proposed method is examination of polymorphism X region of the protein A gene (Spa) by PCR method. This region is one of the distinguishing factors and different patterns of this gene have been identified in various studies. Typing the isolates of S. aureus using Spa gene can be a useful method for epidemiological studies.

Methods & Materials: In this study, 115 samples of S. aureus isolated from human infections after culture on blood agar and mannitol salt agar and catalase and oxidase tests were examined by PCR.

Results: PCR method using Sau primers showed that 103 (89.6%) out of 115 isolates revealed as S. aureus. Isolates for mecA gene were 96 (93.2%) positive and 7 (6.8%) negative. Ninety one (88.3%) and 12 (11.7%) were positive and negative for Spa gene respectively. A total of 86 (85.3%) were positive for both genes. Ten (8.9%) samples were positive for mecA gene, not for Spa gene, 5 (4.8%) were negative for mecA gene, and 2 (1.9%) were negative for both genes.

Conclusion: All differences between the groups using nonparametric chi-square test were significant (P=0.04). In general conclusion, this study showed that most MRSA have virulence genes such as Spa and play a critical role in nosocomial infections.
Meningococcal pneumonia in Japan: A case report and review of the literatures

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Background: Although previous report showed that Neisseria meningitidis (N. meningitidis) was detected from oral cavity of healthy Japanese volunteers, pneumonia caused by this pathogen is very rare in Japan. Here, we present a case of non-invasive meningococcal pneumonia and review case reports in Japan.

Methods & Materials: We searched Japanese case reports of meningococcal pneumonia with Pubmed and the search engine operated by Japan Medical Abstracts Society. The data including the present case were pooled into the following categories for analysis: age, sex, co-morbidities, travel history, symptom, present of bacteremia, patterns of chest images, treatment, prognosis, and serotype of N. meningitidis.

Results: We found 15 cases in the 9 literatures published between 1984 and 2015. The median age of the patients was 42.0 years (range: 18 to 78 years), and men were predominant (68.8 %, 11 of 16 patients). The most common underlying condition was respiratory diseases (50.0 %) such as asthma, chronic obstructive pulmonary disease, interstitial pneumonia, and diffuse panbronchiolitis. The second most underlying disease was mental disorder (25.0 %). Two patients (12.5 %) had a travel history. Fever, cough, dyspnea, disturbance of consciousness, and chest pain were noted in 68.8 %, 62.5 %, 25.0 %, 12.5 %, 6.3 %, respectively. Blood cultures were positive in 2 of 16 cases (12.5 %), but no patient developed meningococcemia despite the present of bacteremia. Bilateral chest infiltration was observed in 2 cases (18.2 %), and right- and left-sided pneumonia were 7 (63.6%), 1 (9.1 %), respectively. Beta-lactams were used in 11 cases (78.6 %). All cases were cured with appropriate antibiotics. Serogroup B meningococci were identified in 5 cases (31.3 %). There were 3 cases of nosocomial transmission and one case of intra-familial infection.

Conclusion: In our study, there was no meningococcal pneumonia with meningitis. All cases were recovered by prompt and appropriate treatments. We reconfirmed that meningococcal pneumonia was very rare in Japan, particularly after 2009. Since national survey of invasive meningococcal disease has started from April 2013 in Japan, the case report of meningococcal pneumonia might be increased in the future.
Phenotypic and genotypic characterization of V. cholerae O1 strains isolated in Democratic Republic of Congo in sanctuaries areas

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Background: Cholera is the most severe of acute diarrhea epidemic caused by V. cholerae. It is classified into two biotypes: classic and El Tor, and three serotypes: Inaba, Ogawa and Hikojima. The first six cholera pandemics were caused by the classical biotype. In 1961, El Tor replaced the classical biotype upon occurrence of the seventh pandemic. Since the last decade, a new variant of El Tor has been documented. The project objective was to investigate the V. cholerae strains circulating in sanctuaries sites in DRC.

Methods & Materials: Phenotypic and genotypic characterization of ten strains of V. cholerae O1 from Kalemie and Uvira isolated in 2014 and 2015 were investigated by classical bacteriological techniques, PCR and PFGE.

Results: These strains belong to the El Tor biotype and serotype Ogawa. The presence of ctxB1 virulence gene suggests the existence of a variant of El Tor. Strains from Kalemie presented four antibiotic resistance (colistin, nitrofurans, nalidixic acid and Norfloxacin) whereas Uvira resistance was observed for seven antibiotics (colistin, nitrofurans, nalidixic acid, Norfloxacin, Sulfonamides, Streptomycin, Sulfonamides and trimethoprim Qunolone resistance and mutations on gyrA and parC genes were observed. The molecular genotyping method in this study; the pulsed field coupled to two enzymes Not I and Sfi I allowed to differentiate two different strains circulating in Uvira and Kalemie.

Conclusion: Two different strains circulating in Uvira and Kalemie have been highlighted both by their PFGE profiles and antibiotic resistance.
Clinico-microbiological spectrum of infective endocarditis at a tertiary care centre

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Background: Infective endocarditis (IE) is a microbial infection of the endothelial surface of the cardiac valves. Despite advances it is associated with morbidity and mortality. Rapid diagnosis, effective treatment, and prompt recognition of complications are essential for good patient outcome.

Methods & Materials: The medical records of 191 patients clinically diagnosed with infective endocarditis were reviewed for clinical and microbiology data admitted between January 2011 to Sep 2015. Blood cultures were detected by using BacT/Alert FAN and SN aerobic bottles. 68/191 cases were positive for bacterial pathogens. The isolates were identified and sensitivity was tested using API and Vitek 2 systems.

Results: The age range of the patients were 17-54 years with a female preponderance. Chronic Rheumatic heart disease (CRHD) was the most common predisposing factor followed by valvular abnormalities, mostly mitral stenosis. 24/191 cases had Prosthetic valve endocarditis (PVE), 167/191 had Native valve endocarditis (NVE). 19/24 (79.1%) PVE cases and 49/167 (29.34%) NVE were culture positive. Culture negative endocarditis was 123/191 (64.39%).

Isolates for NVE belonged to the Streptococcus species and include Streptococcus mitis (15), Streptococcus sanguinis (8), Streptococcus pyogenes (3), Streptococcus pneumonia (1), Enterococcus faecalis (5), Enterococcus faecium (6), Nutritionally variant streptococci (2), Gemella morbillorum (2). Methicillin Resistant Staphylococcus aureus (1), Methicillin Sensitive Staphylococcus aureus (2), Brucella melitensis (2), and also includes Brevundimonas diminuta (1), Corynebacterium diphtheria (1).

The isolates for PVE were Methicillin Resistant Staphylococcus aureus (4), Methicillin Sensitive Staphylococcus aureus (2), Methicillin Resistant coagulase negative Staphylococcus (7), Klebsiella pneumonia (3), Achromobacter denitrificans (1), Burkholderia cepacia (2).

The NVE were treated with a combination of a B-lactam or glycopeptide with an aminoglycoside intravenously for prolonged period of 4-6 weeks with a successful outcome. The PVE cases were treated with the appropriate antibiotics as per the antibiotic susceptibility report.

Conclusion: Despite recent advances, the management of IE remains a serious and challenging problem. The high morbidity and mortality rates, accurate identification of aetiological agents and appropriate antimicrobial therapy are associated with IE. Strict infection control measures will help to reduce the incidence of PVE, which is most often due to hospital acquired pathogens.
Risk factors associated with persistence of staphylococcus aureus bacteremia

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**Background:** *Staphylococcus aureus* bacteremia (SAB) is one of the serious nosocomial or community acquired infections. SAB can persist longer as compared to bacteremia with other bacteria, although whether risk factors associated with persistent SAB which pointed out in previous studies are related to its persistency of SAB in the settings with different antibiotic resistant rate and different antibiotic use. In this study, we determined clinical characteristics and risk factors for persistent SAB by comparing persistent SAB cases and non-persistent SAB cases in Japan.

**Methods & Materials:** All the first episodes of adult SAB cases in 1,150 bed academic medical hospital in Japan from May 2009 through April 2014 were enrolled. The onset of SAB was defined as the time when the first positive blood cultures were collected. Persistent SAB was defined as a case in which positive blood culture persisted 72 hours or longer, and non-persistent SAB was defined as a case negativity of blood culture was verified within 72 hours. Clinical backgrounds, primary infection sites, methicillin resistant or not (MRSA or MSSA), vancomycin susceptibility, and antibiotic use were retrospectively reviewed from medical records.

**Results:** Of 618 SAB cases, MRSA cases and MSSA cases were 293 and 325 cases, respectively. Persistent SAB were 42 cases, non-persistent SAB were 34 cases. Median persistent periods of persistent SAB and non-persistent SAB were 2 days and 8 days, respectively. Clinical backgrounds and primary infection sites primary infection sites were similar between the two groups. The rate of MRSA was in persistent SAB was statistically higher than that of non-persistent SAB (93% vs 35%, P<0.001)(Fig.1). Although susceptibilities to vancomycin, were similar between the two groups, the timing of susceptible antibiotics use in persistent SAB was later than that in non-persistent SAB (2 days vs 0 days, P <0.001) (Fig.2).

**Conclusion:** In our study, persistence of SAB were associated with MRSA as a pathogen, delay in susceptible antibiotic use, but not with clinical backgrounds nor with vancomycin susceptibilities.
Comparison of the outcome of Clostridium difficile infection between patients treated with metronidazole and patients treated with vancomycin: A multi-center retrospective cohort study in Japan

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Background: The rising incidence and worsening severity of Clostridium difficile infection (CDI) have prompted improvements in the treatment of CDI in many countries. Vancomycin (VCM) and Metronidazole (MNZ) are both widely used. VCM may bring about a better clinical response and outcome than MNZ in treating CDI, especially severe CDI. The optimal treatment for CDI, however, has yet to be established in Japan. Dosages and durations of medications for CDI have not been clearly described in previous studies, and MNZ has only recently been approved for CDI under the national health insurance system. Our group conducted Japan's first multi-center retrospective study to investigate the optimal treatment for CDI by comparing outcomes between MNZ- and VCM-treated groups at variable dosages and treatment durations.

Methods & Materials: CDI patients hospitalized at four teaching hospitals from April 2012 through September 2013 were enrolled. CDI was diagnosed when CD toxin was positive by enzyme immunoassay test in stool. The patients were treated for 10-14 days with oral MNZ (1000 or 1500 mg per day: MNZ group) or oral VCM (500 mg per day: VCM group). Kaplan-Meier curves were created to compare the survival curves between MNZ- and VCM-treated patients.

Results: We tested 3,921 stool samples and identified 170 patients with CDI (1.04 cases per 10,000 patient-days). Seventy-eight of the CDI patients were treated with MNZ and 41 were treated with VCM. No significant demographic or clinical differences were found between the two groups. The 90-day all-cause mortality rates in the MNZ and VCM groups were 16.2% and 28.9%, respectively. The survival curves did not differ significantly between the two groups after adjusting for several confounders established to be independent risk factors for severe or complicated-course CDI (age, hypoalbuminemia, acute kidney injuries, leukocytosis, hypotension or shock).

Conclusion: Our study, Japan’s first multi-center assessment of CDI treatment, showed no significant difference in prognosis between the MNZ group and VCM group. We expect MNZ to become a drug of choice for the treatment of CDI.
Clinical profile, susceptibility patterns, treatment and outcomes of melioidosis in India

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Background: Melioidosis, caused by Burkholderia pseudomallei, is endemic to India and has been associated with significant morbidity and mortality. Since it mimics several other diseases, it is grossly under-recognised. This study was undertaken to describe the clinical manifestations, drug susceptibility and outcomes of melioidosis in India.

Methods & Materials: We carried out a retrospective study of adult patients admitted with culture proven melioidosis in a tertiary care hospital in South India from 2008 to 2014.

Results: 114 patients, with a mean age of 45 years (92% males) were included. The patients were from 15 states, majority being from West Bengal (26.3%), Jharkhand (22.8%) and Tamil Nadu (14.9%). Common risk factors included diabetes (82.3%), and alcoholism (14%). The mean duration of symptoms, commonly fever, was 4 months and majority (78%) had multifocal disease. Patients presented in the cooler months (80.5%), especially in the acute group. Chronic melioidosis was commoner than acute disease (64% vs 36%). 11 patients (15%) among chronic melioidosis presented with an acute worsening and bacteremia. Bacteremia (80% vs 41%) and respiratory involvement (39% vs 16%) were more common in acute disease. Chronic granulomatous disease was associated with splenic (50% vs 29%), genitourinary (17.8 vs 4.9%) and bone involvement (12.3 vs 7%). Drug susceptibility to Carbenenems was 100%, Ceftazidime 98.1% while resistance to Trimethoprim-Sulfamethoxazole and Doxycycline was 5.9% and 2.6%. Our patients received induction therapy with Ceftazidime or Meropenem followed by eradication treatment with Trimethoprim-Sulfamethoxazole and Doxycycline. 50.9% patients required a surgical intervention and 21.9% were admitted to an intensive care unit. The case fatality rate was 14.9%. Bacteremia (p <0.001) and respiratory involvement (p=0.003) were associated with increased mortality. 57.9% patients were followed up and 3.5% patients had a relapse.

Conclusion: Melioidosis is an emerging infection in India. Majority of the patients are diabetics presenting with chronic granulomatous disease. Patients with septicemia and respiratory involvement had poorer outcome. A high index of suspicion in the appropriate clinical setting, and early initiation of therapy are essential.
Emerging trends in the antibiotic susceptibility pattern of vibrio cholerae in North Karnataka

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Background: Acute diarrhoea is the second most prevalent communicable disease and a fourth leading cause of death in India with 10762500 cases and 32218 deaths reported in 2013. Cholera is an acute diarrhoeal illness caused by toxigenic strains of Vibrio cholerae serogroups O1 and O139. This study has been done with the aim of evaluating the serogroups, antimicrobial susceptibility pattern, age and gender wise distribution of Vibrio cholerae isolates from cholera epidemics in Bidar and rural parts of north Karnataka, India during the years 2008 to 2015 till date.

Methods & Materials: A total 500 stool samples of cholera outbreak from Year 2008 to 2015 till date were collected and processed at Department of Microbiology, Bidar Institute of Medical Sciences, Bidar, Karnataka, India as per the routine microbiological investigations. The isolates were identified as Vibrio cholerae and confirmed by serological tests with Polyvalent O1, O139 and mono specific Ogawa and Inaba antisera. Antibiotic sensitivity testing was done as per the CLSI guidelines.

Results: Vibrio cholerae biotype ElTor, sero group O1 grown in 155 samples (31%). Among 155 isolates obtained, of which, 136 (87.74%) were belonged to Ogawa, 13 (8.3%) belonged to Inaba and 06 (03.8%) to Hikozima. The isolates showed Multi drug resistance to Ampicillin (81.93%), Nalidixic acid (66.45%) and Ofloxacin (32.90%) throughout the study period. And Vibrio cholerae isolates showed resistance to Ciprofloxacin (22.58%), Doxycycline (14.83%) and Tetracycline (16.77%) indicating development of resistance of V. Cholerae to the drugs which are commonly used to treat Cholera infection. The infection was predominant in male and among patients age between 0-10 years (26%).

Conclusion: This is the first study conducted in the North Karnataka which reflects the importance of control and monitoring of V. cholerae by serogroup and antibiogram typing for policy makers and health professionals of this region as incidence of cholera increased year wise and changing trend in the antibiotic susceptibility pattern of V. cholerae was observed which is due to environmental factors and widespread use of antibiotics.
Bacteriological profile of chronic osteomyelitis in a tertiary care hospital in South India

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Background: Chronic osteomyelitis (COM) is a major medical problem in most countries, mainly associated with violent trauma, modern surgery and inadequate treatment of acute osteomyelitis. Although the incidence of osteomyelitis has reduced to a certain extent with the advent of antibiotics and chemotherapeutic agents, yet it continues to be a major problem in India.

Methods & Materials: A retrospective analysis of data of purulent (Pus/pus swabs, tissue) specimen received from patients with chronic osteomyelitis by the microbiology department between January 2013-October 2015 was carried out. The samples were processed using standard microbiological techniques. Identification and antimicrobial susceptibility pattern of the bacterial isolates were done using the Vitek 2 (bioMerieux, Marcy l'Etoile- France) system.

Results: In all, 184 patients with chronic osteomyelitis were documented during the study period. There was a male preponderance (163 / 184, 88.5%) with majority, in the age group of 10-20 years. Trauma was the major risk factor for osteomyelitis (95/ 184, 51.6%). The lower limb bones were more commonly affected of which femur (166/184, 90.2%) was the predominant bone involved. Culture was positive in 104/184 (56.6%), with the Gram positive organisms most predominant. MRSA was the predominant organism isolated in 28 /104 (27%) cases. Among Gram negative bacilli, Escherichia coli was most common organism isolated in 11/104 (10.4%). Other organisms isolated included Pseudomonas aeruginosa, Klebsiella pneumonia, Acinetobacter baumanii, Proteus mirabilis, Enterobacter cloacae, Morganella morgagnii and showed a high level of antibiotic resistance. In this study no anaerobic organisms were isolated. One patient had a mixed infection with Mycobacterium tuberculosis and A.baumannii. Majority of the patients (85/184, 46%) were managed conservatively with wound care and antibiotics. No mortality was recorded.

Conclusion: Prognosis of chronic osteomyelitis depends on proper microbiological techniques that help in isolation, identification and treatment of the bone-infecting, often multidrug resistant organism. Complications can be further reduced with surgical debridement and removal of the dead tissues.
Characterization of diarrhoegenic escherichia coli using a novel multiplex PCR
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Background: Diarrheal diseases are the second most common cause of infant mortality and morbidity in children less than five years of age. Among all the diarrheal pathogens, Diarrhoegenic Escherichia coli (DEC) plays an important role in epidemic and endemic diarrhea. For the identification of these pathogens, serotypic markers may correlate but are rarely reliably sufficient in identifying a strain as diarrheagenic. PCR targeting the virulence factors is a reliable and rapid way of detecting DEC. This study was undertaken to characterize DEC with diarrhea using multiplex PCR DEC and to obtain the clinico-microbiological profile of the infection.

Methods & Materials: One hundred and twenty children below 5 years of age with diarrhea attending Paediatric department were enrolled in our study after obtaining written informed consent of their parents or guardians for a period of two years from October 2013 to October 2015. Fecal samples were collected from these children. Escherichia coli isolates grown were identified by biochemical reactions. A multiplex PCR was developed to detect the virulence genes (aggR, eaeA, bfpA, ial, lt, st, stx1 and stx2) of various pathotypes of DEC.

Results: Among all, 45 samples had DEC. Of them 21(49%) were Enteropathogenic E.coli, 6(13.3%) were Enterotoxigenic E.coli, 5 (11%) were Enteroaggregative E.coli, 4(9%) were Enterohaemorrhagic E.coli and 3(6.6%) were Enteroinvasive E.coli. We also found in our study 3(6.6%) hybrid strains (2 were atypical EPEC and EAEC and 1 was EIEC with EHEC) and 3(6.6%) mixed infections {EAEC and EIEC, typical EPEC and EHEC, EAEC and atypical EPEC). Out of 22 EPEC positive samples, 5 (22% ) were identified as atypical EPEC as they possessed eaeA only and not bfpA, 3 (13.6%) isolates were identified as typical as they possessed both eaeA and bfpA genes, while 13 isolates(59%) possessed only bfpA gene. The majority (90%) of cases of EPEC diarrhea clustered in the less than two years age group.

Conclusion: This multiplex PCR technology will serve as a good epidemiological tool for the screening of pathotypes of DEC that causes diarrhea in children.
Conclusion
Spontaneous bacterial peritonitis in patients of cirrhosis of liver with ascites
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Background: Spontaneous bacterial peritonitis (SBP) is a common and fatal complication occurring in cirrhotic patients with ascites. It is defined as infected ascites in the absence of any recognisable secondary cause of infection. This study was undertaken to find out the rate of occurrence of SBP in patients of cirrhosis with ascites, to find out relative frequency of variants of ascitic fluid infection, to study clinical presentation and laboratory profile and to determine relationship between MELD score and the occurrence of SBP.

Methods & Materials: This hospital based cross-sectional study was carried out in a tertiary care hospital in Nagpur after taking approval from Institute's Ethics Committee. 100 patients of cirrhosis with ascites irrespective of age and gender were enrolled after taking their written informed consent. 20 ml of ascitic fluid was aspirated in heparinised disposable syringe; out of it 10 ml was immediately inoculated into blood culture bottle at bedside and sent for bacterial culture along with the remaining 10 ml for routine biochemical and cytological examination.

Results: Majority of the patients were between 40-49 years of age, mean age of patients diagnosed as SBP was 42.51 years. Out of total 42 cases of SBP, classical SBP was present in 16 (38.09%), Culture negative neutrocytic ascites in 14 (33.33%), Bacterascites in 12 (28.57%) patients with SBP. Escherichia coli was the most frequently cultured organism isolated in 15 cases (53.57%), followed by pseudomonas in 9 (32.14%), Klebsiella pneumoniae in 3 (10.71%) cases. The common mode of presentation of SBP was abdominal tenderness (65.38%) followed by hepatic encephalopathy (58.82%) associated with abdominal pain (50%) and fever (46.66%), distention of abdomen (44.30%) hematemesis and melena (45%). Hyponatremia with a serum was found to be associated with severe complications. In (52.63%) patients of SBP ascitic fluid protein was less than 1 mg/dl. MELD score was found to be a reliable index of disease severity.

Conclusion: SBP is a fatal complication of cirrhosis with ascites. It has heterogenous clinical presentation. Ascitic fluid should be analysed routinely in all cases of cirrhosis with ascites for the early detection of SBP.
Tropical pyomyositis - outcomes and clinical profile

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Background: A classical tropical disease, Pyomyositis is a primary suppurative bacterial infection involving the skeletal muscles due to hematological spread, presenting with pain and inflammation in the involved muscles. Incidence is more in an immunocompromised host, with trauma, injection drug use being common predispositions. *Staphylococcus aureus* is the common inciting agent. This study was undertaken to characterise the clinical profile and outcomes of the disease due to a dearth of data from India.

Methods & Materials: Methods - Retrospective study was conducted on 66 patients admitted with the diagnosis of primary pyomyositis between January 2013 to January 2015.
Inclusion criteria - Patients with pyomyositis
Exclusion criteria - Secondary infection, concomitant febrile illnesses, viral myositis
Primary outcome - Response to antibiotic or surgical therapy, relapse or death.

Statistical analysis - Using SPSS 21. Normally data is presented as mean ± standard deviation.

Results: The mean age was 37.61 ± 22.25 yrs. Among presenting features, myalgia was in 98.5% patients, fever in 78.8% patients. 54.5% manifested local signs of inflammation and tenderness was in 90.9% patients. With 24.2% of patients having uncontrolled sugars, Diabetes was the most common risk factor. Trauma was present in 18.2% and 3% were on steroids.
Iliopsoas was involved in 59.1% patients, Quadriceps in 13.6% patients, Hamstrings in 7.5%. Single muscle was involved in 86.4% of the patients and multifocal involvement in 13.6% of patients.
For diagnosis, Ultrasound was used in 56.01% of the patients, Magnetic resonance in 30.03% and Computed tomography in 13.06% of the patients.
10.6% had Acute Kidney Injury.
Microbiologically, 18.2% patients had Methicillin sensitive *Staphylococcus aureus* positivity in the culture. 12.1% patients had Methicillin resistant *Staphylococcus aureus*. 81.8% of the patients recovered with antibiotics and pus drainage. 6.1% had relapse. 3% of the patients died with sepsis.

Conclusion: Pyomyositis should be considered in the differential in case of a painful swelling in a muscle in an immunocompromised.
Ultrasound should be used as an adjunct in the diagnosis.
Empiric therapy covering *Staphylococcus aureus* is prudent pending culture reports.
Virulence gene profile and SCCmec types of clinical MRSA isolates: Is there a fitness cost involved?

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Background: The success of methicillin resistant Staphylococcus aureus as a pathogen is attributed to its extraordinary repertoire of virulence factors. Based on SCCmec types, MRSA isolates can be classified as hospital (HA-MRSA) or community acquired (CA-MRSA). Association of certain virulence genes with particular SCCmec types has been previously reported. For instance, pvl gene is associated with SCCmec types IV or V (CA-MRSA types).

Methods & Materials: Two hundred non-repetitive isolates of MRSA from clinical specimens were screened for virulence genes such as pvl, tsst, hlg, enterotoxin A (sea), exfoliative toxin A (eta), intercellular adhesion (ica) genes by PCR. SCCmec typing was carried out by multiplex PCR. An attempt was made to find the association between virulence genes and the clinical presentation as well as with the SCCmec types to establish the fitness cost, if any.

Results: A total of 192 isolates (96%) carried one or more virulence genes while 8 (4%) had none. The commonest virulence gene encountered was ica (90%) followed by hlg (83%), sea (78%), pvl (53%), eta (12%) and tsst (2%). Out of 200 MRSA isolates, only 40% carried single SCCmec type, whereas 59% carried multiple SCCmec types including a combination of classical HA and CA types. The predominant SCCmec type found in our study was III followed by SCCmec V. eta and pvl toxins were mainly encountered in isolates from severe skin and soft tissue infections whereas the isolates which were negative for the virulence genes tested were obtained from mild skin infections. Notably, all the blood isolates (n=12) were negative for pvl and eta, whereas all were positive for hlg. pvl gene positivity was significantly associated with SCCmec type V followed by type III. Majority of the blood isolates and all the tsst positive MRSA isolates carried SCCmec III. The single isolate with SCCmec type II was negative for all the virulence genes tested.

Conclusion: This study documented the presence of virulence genes in various combinations in majority of the clinical MRSA isolates. No strong evidence for fitness cost of SCCmec was established as the isolates negative for virulence genes belonged to diverse SCCmec types.
The human microbiome research in Africa – A systematic review
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Background: The explosion of interest in the human microbiome research was sparked in part by two research initiatives launched in 2008, the European and Chinese-led Metagenomics of the Human Intestinal Tract Consortium and the National Institutes of Health (NIH)-sponsored Human Microbiome Project. The new knowledge provided by these initiatives and others worldwide are transforming our understanding of the pathogenesis of several diseases, including asthma, inflammatory bowel diseases, obesity and Type 1 diabetes, as well as the development of the immune response linked to some vaccines. However, African populations are understudied in these initiatives, and there is no “African Microbiome Initiative”. This systematic review aimed to summarize and discuss the state of research on the human microbiome in Africa, including the therapeutic role of the microbiome for the management of certain local diseases.

Methods & Materials: Using predefined keywords, we searched in six electronic databases for human microbiome studies conducted in Africa. In addition, to find additional articles, we checked references cited in eligible studies. Two authors independently selected eligible studies published until 30 September 2015.

Results: Eighty-nine human microbiome papers were identified from six electronic databases and other sources. There were 80 primary microbiome studies (including Khoi San (n = 1) and Hunter-Gatherers (n = 4)) and 9 nested microbiome studies within existing cohorts. 16S rRNA gene sequencing was the technique most widely used to characterize the microbiota. The main body sites studied were the gut (46%), vagina (25%) and the oral cavity (11%). The diseases targeted were malnutrition (n = 4), HIV/AIDS (n = 7), diarrhoea (n = 4), and periodontitis (n = 5). These microbiome studies were performed in individuals of all ages, with most of the studies being conducted in adults. Kenya, Uganda, South Africa and Nigeria were the sites of the majority of studies; however the principal investigators of most of the studies (87%) were from developed countries. The USA NIH was the main funding source (25%), followed by the Bill and Melinda Gates Foundation (9%) and the European Commision (8%).

Conclusion: More studies of the microbiome including African participants, focusing on endemic diseases and led by African researchers are needed.
Nocardiosis-a clinicoepidemiological profile over 10 years
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Background: Nocardiosis is a clinical and diagnostic challenge, compounded by lacunae in existing literature. Our objectives were to establish the clinical spectrum of this disease in our setting, describe the most common causative agent of the disease and to ascertain differences in our patient population from available data.

Methods & Materials: This was a 10 year (2004-2013) retrospective study carried out at a tertiary care centre in South India, of 131 cases of nocardiosis. The electronic medical records were studied and data analysed.

Results: Sixty three percent were male, 23% of all in the sixth decade of life. The most common sites of infection were the skin and the eye -36 (27%) patients each and the lower respiratory tract -35 patients(26%). 48 (37%) patients were on immunosuppressant therapy, either a triple drug therapy following renal transplant, autoimmune disorders/ haematological malignancies on combination immunosuppressants or patients on prolonged corticosteroids. Of 36 patients with nocardiosis of the eye, 30 (83%) were corneal ulcers with history of trauma with vegetative matter or soil, and 5(14%) were endophthalmitis following intraocular lens implantation. 16(46%) patients with respiratory tract nocardiosis had a previous lung pathology. 11(8%) were HIV associated nocardiosis. Disseminated disease was seen in 7(5.3%) patients following renal transplant and in 3(2.3%) patients with SLE, all on triple drug immunosuppression. The most common organism isolated was Nocardia asteroides in 73(56%), followed by Nocardia spp in 32(24%), aerobic actinomycetes in 24(18%) and Nocardia brasiliensis in 2(1.5%). All patients responded to treatment with cotrimoxazole alone or in addition to surgical debridement for cutaneous and subcutaneous lesions. There was only one Nocardiosis related death in this cohort of patients. Antimicrobial susceptibility testing performed on 72 isolates showed 6.9%, 9.7%, 31%, 38%, 75%, 42%, 31%, 74% susceptibility to penicillin, ampicillin, erythromycin, tetracycline, cotrimoxazole, chloramphenicol, cefazolin and triple sulfa respectively.

Conclusion: We report a predominance of nocardiosis from the eye and nocardiosis following immunosuppression. The most common species isolated was N.asteroides. A paucity in HIV associated nocardiosis is striking. Antimicrobial susceptibility showed 75% susceptibility to cotrimoxazole, the drug of choice, which was reflected by a good response to therapy in this cohort.
Cholera outbreak investigation, Gajala community, Birnin Kudu Local Government Area (LGA), Jigawa State, Nigeria, September 2015

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Background: Outbreaks of cholera are common in Nigeria, at week 40 this year, it has occurred in 18 states (95 LGAs). Overall, 4542 cases has been recorded, case fatality rate (CFR) 3.87%. An outbreak of cholera was reported in Gajala community, Birnin Kudu Local Government Area, Jigawa State, Nigeria on September 16th, 2015. We carried out an investigation to confirm, characterize, assess the magnitude of the outbreak and identify possible risk factors.

Methods & Materials: We carried out descriptive characterization of the outbreak, active case search and an un-matched (1:2) case control study. A case was defined as “Any person ≥2 years living in Gajala community, presenting with acute watery diarrhea, with or without vomiting from September 11th to 25th 2015”, and a control defined as above but without acute watery diarrhea or vomiting. Median age of the 138 respondents was 20 years (range: 2-7). Frequencies, univariate, bivariate and multivariate analysis was done using Epi info version 3.5.3. Stool samples from case patients was tested with rapid diagnostic test (RDT) kit (smart kit) and water samples cultured in the laboratory. Finally, we assessed the sanitary and waste disposal system.

Results: We recorded 50 cases, median age 21 years (range: 2-80), more male were affected, 27(54%), attack rate (AR) 10.5%, one death occurred, CFR 2%. Mean duration of illness before seeking healthcare was 17.2 hours (SD: 15.2). Stool samples tested positive to Vibrio cholerae RDT, water samples yielded negative result. The significant risk factors were not washing hand before eating, (age adjusted odds ratio (AAOR): 2.78 (1.16-6.63), not washing hand after toilet, (AAOR: 2.63 (1.18-5.85), and poor knowledge of cholera (AAOR: 2.52 (1.23-5.28). Good sewage system (pit toilet covered) and proper waste disposal system was observed.

Conclusion: Cholera outbreak in Gajala 11th-25th September 2015, was caused by Vibrio cholerae of serotype 01, and characterized by severe illness, high AR and CFR. Poor knowledge of cholera and hand hygiene practice likely caused the outbreak. Public health education on cholera and hand hygiene assisted to stop the outbreak. We recommended continued health education, improvement in water supply, emergency preparedness and maintenance of surveillance.
Clostridium difficile infection at outpatient clinic without known risk factors - CDI can be antibiotic-unassociated diarrhea in outpatient setting

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Background: Clostridium difficile is a common cause of antibiotic associated diarrhea. Besides use of antibiotics, there are a number of risk factors associated with Clostridium difficile infection (CDI). Nosocomial exposure during hospitalization is one of such risk factors. However, it has been reported that community-onset, healthcare facility–associated disease (CO-HCFA) and community-associated CDI (CA-CDI) are reasing in Europe and North America. However, the incidence and characteristics of CO-HCFA and CA-CDI in Asian countries are not well known.

Methods & Materials: We performed a retrospective chart review on community onset or associateed CDI at a Japanese primary care clinic from January 2014 to September 2015. Cases with positive stool CD toxin test with clinical diagnosis made by Board certified gastroenterologists were defined as CDI. CO-HCFA, CA-CDI and severity of disease were defined as according to the SHEA –IDSA guideline. History of recent antibiotic exposure within 1 year was also evaluated. Age, sex, statin and gastric acid suppression drug use (i.e. proton pump inhibitor or H2 blocker), and Charlson Comorbidity Index (CCI) Score were also assessed. Patient’s occupational exposure to healthcare setting were also assessed.

Results: Four cases of CDI were diagnosed during study period. Every patients presented with chief complaints of diarrhea and fever. All of them were CA-CDI, and nobody had exposure to healthcare. Patents’ ages range from 15 to 47 with one being woman. One case had the history of previous antibiotic use within one year. All the cases were mild to moderate disease. All the patients’ CCI score were 0 and nobody used gastric acid suppression drugs.

Conclusion: None had known other risk factors associated with CDI, which suggests CDI should be within differential diagnosis of community onset diarrhea in Japan. Since all our cases are mild to moderate, there might have been undiagnosed CDI which resolve spontaneously. Further studies may be necessary to evaluate true incidence and clinical significance of CA-CDI in our settings.
Salmonella manipulation of host signalling pathways promotes cellular transformation and cancer of infected tissues


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Background: Cancer arises through a multistep process, fuelled by deregulation of pathways in control of cellular growth and proliferation. The very same signalling cascades are targeted by infectious pathogens en route to subversion of the host to their benefit. One such pathogen—the bacterium Salmonella Typhi—is epidemiologically associated with an increased incidence of gallbladder carcinoma, as both are highly prevalent in India while relatively rare in the Western world.

Methods & Materials: The present study demonstrates that Salmonella infection induces stable cellular transformation in pretransformed gallbladder organoids and mice. Inactivation of the TP53 pathway in combination with c-MYC oncogene amplification is minimal prerequisites for Salmonella-induced transformation in cell culture. Such alterations are also found to be prevalent in gallbladder carcinoma samples from India, relative to those derived from patients in the Netherlands.

Results: In the infection process, Salmonella injects target cells with effector proteins that firstly activate the host’s MAPK cascade during bacterial uptake, and secondarily turns on the AKT pathway to ensure intracellular bacterial survival. We found that both signalling pathways are activated in gallbladder carcinoma samples collected in India and are critical for maintaining the transformed state induced by Salmonella infection in cell culture.

Conclusion: Collectively, our findings suggest that by virtue of manipulating host signalling for its own purposes, Salmonella promotes transformation in genetically predisposed cells as collateral damage, thus contributing to cancer in infected tissues, such as gallbladder.
Factors associated with Urinary tract infections caused by extended spectrum β-lactamase (ESBL) producing organisms in Sri Lanka
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**Background:** Urinary tract infections (UTI) caused by extended-spectrum β-lactamase (ESBL)-producing organisms are a major burden in clinical practice. Hospitalization in the past 3 months, antibiotic treatment in the past 3 months, age over 60 years, diabetes mellitus, Klebsiella pneumoniae infection, previous use of second or third-generation cephalosporins, quinolones or penicillins are known associations and risk factors for ESBL-UTI.

**Methods & Materials:** A descriptive study was conducted over a period of 6 months from January - July 2015 recruiting patients with UTI caused by ESBL producing organisms, who were admitted to the Professorial Medical unit, Colombo North Teaching Hospital, Ragama Sri Lanka in order to identify risk factors and associations. Data were obtained using a pre-tested interviewer administered questionnaire and from relevant medical records after obtaining informed written consent.

**Results:** 52 patients were recruited; males 30 (57.7%), mean (SD) age 64.1 (.12.6) years. Of them, 46 (88.5%) had diabetes mellitus, 32 (61.5%) had hypertension and 10 (19.2%) had chronic liver disease as comorbidities. 20 (38.5%) had ultrasonographic evidence of acute pyelonephritis. At presentation 16 (30.8%) had biochemical and/or ultrasonographic evidence of chronic or acute on chronic kidney disease. History of constipation was observed in 18 (34.6%), hospitalization during the past 3 months was seen in 24 (46.2%) and history of urinary catheterization in 16 (30.8%). Features of obstructive uropathy such as hydronephrosis, hydrouretre and prostatomegaly were seen in 4 (7.7%) patients each. Antibiotic treatment within the past 3 months was observed in 32 (61.5%); penicillins in 18 (34.6%), 3rd generation cephalosporins in 16 (30.8%), quinolones in 14 (26.9%) and 2nd generation cephalosporins in 12 (23.1%). 18 (34.6%) had received more than one antibiotic within the past 3 months. 8 (15.4%) patients studied were on prophylactic antibiotics for recurrent UTIs. None of them had recent Klebsiella pneumonia.

**Conclusion:** Similar to other studies, diabetes mellitus, recent antibiotic treatment, hospitalization and catheterization were observed in our patients with ESBL-UTI. The fact that only 53.8% patients had received antibiotics at community level and 38.5% patients had never received antibiotics prior to developing ESBL-UTIs suggest high prevalence of ESBL producing organisms at community level.
Pathogenetic significance of macrophage inflammatory protein-IA in patients with erysipelas of the lower extremities
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Background: The aim of study was to clarify the biochemical mechanisms of severe hemorrhagic forms of erysipelas and find the possible early predictors of its development.

Methods & Materials: 90 patients with erysipelas of the lower extremities (ELE) were examined. 44 patients had erythematous (1th group), 24 - bullous (2th group) and 32 - hemorrhagic (3th group) forms of ELE. The level of macrophage inflammatory protein-1A (MIP-1a) was investigated in the dynamics of ELE. Also we studied the phagocytic activity of neutrophils and monocytes.

Results: In the acute phase of disease the level of MIP-1a in 1th and 2th groups was lower than in healthy individuals: 9.10pg/ml (95%CI 7.84-10.36pg/ml), 10.8pg/ml (95%CI 9.43±12.17pg/ml) and 13.6pg/ml (95%CI 11.8±15.32pg/ml) accordingly, p-value 1-n=0.03, p-value 2-n=0.04. In 3th group it was significantly higher in comparison with all other groups 290.7pg/ml (95%CI 284.49-296.91pg/ml). Phagocytic activity was considerably higher in patients of 3th group than in patients of 1th group: 92.5% (95%CI 91.88-93.12%) and 83.5% (95%CI 82.49-84.51%) for neutrophils (p-value 1-3=0.001) and 88.5% (95%CI 87.94-89.06%) and 80.0% (95%CI 77.77-82.23%) for monocytes (p-value 1-3=0.002). In the convalescent period the level of MIP-1a increased to 11.7 pg/ml (95%CI 10.34-13.06pg/ml) at erythematous form and 13.5% (95%CI 9.10-16.74 pg/ml) at bullous form without difference between them and normal level. In 3th group it was down to the level of a norm 13.7 pg/ml (95%CI 10.46-16.94 pg/ml). Within 12 months of follow-up period we observed only 1 relapse in 3th group, 3 - in 2th group and 6 in 1th group.

Conclusion: Identified changes in the level MIP-1a reflect its role in the implementation of the phagocytic activity. Mostly it refers to the monocytes, as evidenced by an increase in its level in patients with hemorrhagic form of erysipelas, where their activity was maximal. Significant reduction of relapses in patients with hemorrhagic erysipelas probably connected with increased PA, which can stimulate nonspecific resistance via activation of phagocytosis.
Elevated expression of miR-223 and miR-21 in Helicobacter pylori induced gastric cancer patients

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Background: Numerous studies have implicated that persistent colonization of the stomach by Helicobacter pylori (H. pylori) is strongly associated with development of gastric cancer (GC). Micro-RNAs (miRNAs) are a wide class of small, noncoding RNAs that negatively regulate protein expression at the posttranscriptional level. The multi-dimensional roles of microRNAs in gene regulation and tumorigenesis have prompted to explore their potentials in diagnosis and treatment of various cancers including gastric cancer. However, the expression or function of miRNAs in gastric tissues obtained from normal and cancerous regions in presence/absence of H. pylori has not been fully understood.

Methods & Materials: Cancer tissues and corresponding normal tissues from 40 GC patients were included in this study. Detection of H. pylori was diagnosed by PCR using ureC specific primers. Expressions of miRNAs were studied by real time PCR following standard protocol.

Results: The study revealed that the expressions of both miR-223 and miRNA-21 were significantly higher (P<0.05) in cancer tissues compared to normal tissues. Out of 40 pairs, 14 pairs samples were positive for H. pylori. Both miRNAs expressions were significantly up-regulated (P<0.03) in H. pylori positive than H. pylori negative samples from both normal and cancerous sites.

Conclusion: Significantly higher expressions of miRNAs in cancerous tissues suggest their role in neoplastic transformation. Our results indicated that both miRNAs function as growth-promoters in H. pylori related GC. This may have therapeutic and prognostic implications for management of GC patients. Relationship between miRNAs expression and H. pylori infection encourage investigators for further study.
Clinical spectrum of Aeromonas infections in hospitalized patients

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Background: The genus Aeromonas is a member of the family Vibrionaceae. Aeromonas hydrophila is the most commonly isolated species associated with human infections. Aeromonas species are gram negative, oxidase positive, non fermenting rods found in fresh water and are known to cause gastroenteritis, cellulitis, necrotizing fasciitis, meningitis, bacteremia and urinary tract infections.

Methods & Materials: Clinical and microbiological profile of twelve patients with Aeromonas infection from a tertiary care hospital from Chennai between April and October 2015 were reviewed. The isolates were identified with the help of biochemical tests, vitek, MALDI-TOF using standard guidelines.

Results: Twelve patients with positive cultures for Aeromonas were studied: 7 patients [58%] acquired infections in the community and 5 were acquired after 48 hours of hospitalisation. Four [33.3%] had skin and soft tissue infections, 3 [25%] had urinary tract infections, 2 [17%] patients had abscesses and one had peritonitis, thrombophlebitis and spontaneous bacteremia each. 3 [25%] had liver disease and 3 [25%] had diabetes as co-morbidities. No patient gave a history of leech exposure or exposure to natural fresh water bodies. 11 [91.6%] isolates were sensitive to third generation cephalosporins and quinolones and 9 [75%] were sensitive to carbapenems. One isolate was an New Delhi Metallo beta lactamase-1 [NDM-1] producer detected by Xpert Carba R and was sensitive only to colistin and aminoglycosides. All were sensitive to aminoglycosides. 10 [83%] out of 12 isolates were A. hydrophilia species and rest were A. sorbia species. Of the 3 patients with bacteremia, 2 patients died whereas patients with isolates from other sites all survived.

Conclusion: Aeromonas species can cause a wide range of community and hospital acquired infections, most commonly involving skin and soft tissue, without a history of freshwater or leech exposure. Detection of NDM-
carbapenamase in one of the isolates might be an indicator of future serious multidrug resistance in this species.
Clinico - Microbiological study of diabetic foot ulcers
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Background: Diabetes mellitus type 2 is a complex disease affecting all the vital organs in the body. 15% of people suffering from type 2 diabetes mellitus have Diabetic foot ulcers (DFU). Diabetic Foot Ulcers are prone to recurrent and chronic infections which eventually debilitates the health of the patients. Limb loss is a significant risk factor in diabetic foot ulcer especially if the treatment is delayed. The aim of this study is to determine the microbiological profile and effective antibiotic regime for infected diabetic ulcers.

Infected Diabetic Foot Ulcer

Methods & Materials: This study was conducted from clinical specimens taken from 54 patients with diabetic foot ulcers over a 8 month period in Hi Tech Medical college & Hospital in Odisha, India. Clinical sampling was done by first washing the wound by sterile saline solution and making a puncture-aspiration from the base of the wound or by applying a sterile cotton swab to the wound. Thereafter the samples were processed by standard aerobic microbiological techniques. The antibiotic susceptibility pattern was studied by the Kirby-Bauer disc diffusion method.

Results: Of the 54 patients evaluated 31 (57.8%) were male and 23(45.2%) were females. 96 organisms were isolated. From the collected specimens most of the organisms were gram negative. The predominant organism isolated was Pseudomonas Aeruginosa (20.3%) followed by Staphylococcus Aureus (14.81%). Other organisms which were isolated were Klebsella species (12.96%), Escherechia Coli (11.12%). Most of the gram negative isolates were resistant to ampicillin-sulbactam, gentamicin, levofloxacin and gatifloxacin. Pseudomonas aeruginosa showed resistance to most commonly used antibiotics, highest resistance was seen with Ciprofloxacin and Ofloxacin and least with Aztreonam and Imipenem All isolates were sensitive to Imipenem.

Pseudomonas Aeruginosa showing green pigmented growth on agar plate.

Conclusion: India has the largest population suffering from diabetes mellitus type 2 and with appreciably poor economic conditions; this study on microbiological profile and effective antibiotic susceptibility in diabetic foot ulcer infections assumes profound significance. The major concern in managing diabetic foot ulcers is increasing emergence of resistance to antibiotics. The increasing prevalence of multi drug resistance to Pseudomonas Aeruginosa is a cause for concern. Prompt approach to diabetic foot ulcers with appropriate antimicrobial therapy may avert limb amputation especially in the developing world.
Mycobacterial esat-6 like protein alters antigen presentation and mediates intracellular survival in a NO and P38 dependent manner

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Background: Early secretory antigenic target (Esat-6) protein coded by esxA is known for its significant role in virulence and T-cell antigenic determination for Tuberculosis. There are at least 23 such ESAT-6 family proteins encoded in Mycobacterium tuberculosis genome; and many of their functions remains uncharacterized. Here we investigated a mechanism by which a member of Mtb ESAT-6 like family proteins alters antibacterial defense mechanism of macrophages by blocking antigen presentation and on the other hand facilitates bacterial persistence inside the macrophages. 

Methods & Materials: Mtb esat-6 like protein (Esl) gene was overexpressed in Mycobacterium smegmatis (MsmEsl) using shuttle vector pSMT3. The role of MsmEsl in intracellular survival was checked by Colony forming unit (CFU) assay. Nitric oxide production was checked by using Griess reagent and iNOS expression by Realtime PCR, Western bloting and Immunofluorescence microscopy. Expression of MHC-II was checked by Flow cytometry analysis; Realtime PCR and Western blotting. The expression of CIITA which is the major transactivator of MHC-II was checked by western blotting. To check the involvement of MAPK and iNOS in intracellular survival and alteration of antigen presentation, intracellular survival and CIITA expression after iNOS, P38 and ERK inhibition was checked by CFU assay and Western blotting respectively.

Results: MsmEsl showed significantly increased intracellular survival in both THP-1 and RAW264.7 cells after 24h of infection. RAW264.7 macrophages infected with MsmEsl strain showed increased NO production and iNOS expression. Macrophages infected with MsmEsl showed decreased MHC-II expression. Similarly, down-regulation of CIITA (Class II transactivator) was also observed in MsmEsl infected macrophages. Cells infected with MsmEsl showed activation of P38 and ERK. Inhibition of ERK, P38 and iNOS decreased intracellular survival. Furthermore, when P38 and iNOS were inhibited, the CIITA level was increased.

Conclusion: The present study highlights the role of an Esat-6 like protein in intracellular survival and alteration of Antigen presenting molecules in macrophages. It mediated intracellular survival and down regulated antigen presentation components by inducing NO production and P38 activation. Altogether this study can help in a better understanding of Mycobacterial pathogenesis.
Umbilical stump infections in neonates with special reference to MRSA

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**Background:** Annually about 3.3 million neonatal deaths occur around the world. Of these, more than 30% are caused by infection. Some of these infections start as umbilical cord infection called as Omphalitis. The umbilical cord area supports growth of some beneficial (commensals) and harmful microorganisms. Data on the incidence in low-income countries estimate the risk to range between 2 and 77 per 1000 live births in hospital settings, with fatality rates of between 1% and 15% depending on the definition of omphalitis used. Community-based data show infection rates to be 197 per 1000 live births in India. The freshly cut umbilical cord is a prime site of bacterial colonization. Omphalitis is proximally caused by colonization that progresses to local signs of infection including pus discharge, redness, swelling, or foul odor. This study has been undertaken to evaluate the organisms causing umbilical stump infections in neonates and to detect MRSA among these cases in NICU of a Pediatric Tertiary Care Centre Niloufer Hospital.

**Methods & Materials:** Prospective study of neonates diagnosed to have omphalitis admitted in NICU. Discharge from umbilical stump was collected using cotton sterile swab premoistened with normal saline. Two swabs were collected and processing was done in Microbiology Laboratory by standard Conventional methods. The clinical isolates obtained were identified and their Antibiograms determined by Kirby Bauer’s Method.

**Results:** Among the 82 clinical isolates obtained 38 were Gram Positive Cocci and 41 were Gram Negative Bacilli. Isolates were predominately from Premature babies accounting to 48%. Neonates were also found to be associated with other illness like Pneumonia and Meningitis which lead to Sepsis. Out of the 38 Gram Positive cocci 14.65% were identified as MRSA by Oxacillin Disc Diffusion and Cefoxitin E test strip methods. With Conventional PCR, the mecA gene was amplified and determined in the MRSA isolates.

**Conclusion:** Antiseptic practices should be meticulously followed to reduce the incidence of umbilical stump infections.
Molecular serotyping of klebsiella pneumoniae capsular types, sequencing of positives and its epidemiology in the Eastern Cape Province, South Africa

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Background: The bacterial capsule is considered a major virulence factor of K. pneumoniae, with serotype-related variation in severity of infection being observed. Recently, methods for the molecular typing of K. pneumoniae have been developed to supplant the existing serotyping method. Serotypes K1–K6 are more associated with severe respiratory infection and septicaemia in humans than the higher numbered serotypes.

Methods & Materials: A prospective, descriptive study based on laboratory investigations at the Microbiology laboratories from Eastern Cape Province in South Africa. Non-duplicate, randomly selected 112 Klebsiella isolates were collected from three areas representing Eastern Cape from August 2011 to July 2014. Real-time PCR detection of Klebsiella K1, K2 and K5 serotypes cps clusters using LightCycler 2.0 was performed. Genome sequencing of positive isolates was performed and compared with published NCBI data base.

Results: Specimen distribution: Mthatha 71 (63.4%), East London 23 (20.5%), Port Elizabeth 18 (16.1%). Mean age of our patients in this study was 23.9 years with SD 23.9659, with a slight male predominance 58 (51.8%) in the group and 103 (92%) of black population. All K1, K2, K5 positive: 22 (19.6%) with distribution of K1 positive 9 (8%), K2 positive 13 (11.6%), K5 positive 0 and Non K1/K2/K5: 90 (81.25%). ESBL forming Klebsiella species were 45.9 %. MDR Klebsiella were found in 19 (17%). The concordance between our K1 internal positive control with published NTUHK2044 and K2 with KP1158 was 100%.

Conclusion: This is believed to be the first report to demonstrate the seroepidemiology of K. pneumoniae in the Eastern Cape province of South Africa. Serotype K1/K2 comprised 19.6% of the K. pneumoniae strains in this study. We did not detect any K5 serotype in this study. K1 serotype was found in exclusively in respiratory specimens. High antibiotic resistance with ESBLs and MDR in ECP. There was significant difference in the prevalence of K1/K2 isolates among Port Elizabeth and Mthatha.
Effective diagnosis of Hydatidosis (Echinococcosis granulosus) by immunomagnetic bead ELISA technique using paramagnetic nanoparticles

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**Background:** Cystic echinococcosis (CE) is a serious public health problem in sheep-raising regions. Detection of *E. granulosus* antigens is a better immunodiagnostic tool than determination of the antibody level. **Objective:** The present study was conducted to evaluate the role of prepared protoscolex antigen in the detection of the infection through raising anti-*E. granulosus* immunoglobulin G polyclonal antibody (IgGpAb).

**Methods & Materials:** The circulating protoscolex antigen (CPA) used was obtained from lung and liver cysts of sheep and camel and injected in rabbits to raise specific polyclonal antibodies (pAb) against *E. granulosus*. A novel immunomagnetic bead ELISA based on immunoglobulin G (IgG) for detection of CPA in sera of rabbit infected with *E. granulosus* was developed.

**Results:** Detection of CPA in serum by sandwich ELISA gave a sensitivity of 90.48%, a specificity of 91.3%. On the other hand, detection of CPA in serum by sandwich ELISA with paramagnetic nanoparticles gave a sensitivity of 95.2%, a specificity of 95.5%.

**Conclusion:** The novel assay appears to be sensitive for detection of Echinococcosis antigen more than sandwich ELISA technique.
Nano-Gold Sandwich ELISA: A key for G. duodenalis early diagnosis in patient's stool and serum samples of infected patients

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Background: With the ability to interact with matter at the nanoscale, the development of nanotechnology architecture and materials could potentially extend sub-cellular and molecular detection beyond the limits of conventional diagnostic modalities.

Methods & Materials: In this paper, we aimed to detect the potential specificity and sensitivity of nano-gold beads-based-ELISA in diagnosis of giardiasis in stool of asymptomatic and symptomatic infected individuals. Giardia (G.) lamblia antigen in stool samples was detected by using the conjugated anti-purified G. lamblia cysts antigen (PGA) with nanoparticlelessandwich ELISA, compared to the traditional sandwich ELISA.

Results: Sandwich ELISA achieved sensitivity of 93%, specificity of 92.5%, positive predictive value (PPV) of 95.7% and negative predictive value (NPV) of 88%, while nano-sandwich ELISA achieved sensitivity, specificity, PPV and NPV of 95.8%, 95%, 97.2% and 92.6%, respectively.

Conclusion: In conclusion, our research study provides that nano-sandwich ELISA is a well-established reference test for diagnosis of giardiasis.
Dried cerebrospinal fluid spots for diagnosing Japanese Encephalitis Virus (JEV) infection by Anti-JEV IgM antibody capture enzyme-linked immunosorbent assay: Harnessing the potential of a fully saturated pre-cut filter paper disc

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Background: The use of filter paper as a simple, inexpensive tool for storage and transportation of blood, ‘Dried Blood Spots’ or Guthrie cards, is well-established. In contrast, there are a paucity of data on dried cerebrospinal fluid (CSF) spots. This has potential applications in low-resource settings, such as Laos, where laboratory facilities for central nervous system (CNS) diagnostics are only available in Vientiane. In Laos, a major cause of CNS infection is the Japanese encephalitis virus (JEV). We aimed to develop a dried CSF spot protocol and evaluate performance using the World Health Organisation recommended anti-JEV IgM antibody capture enzyme-linked immunosorbent assay (JEV MAC-ELISA).

Methods & Materials: Sample volumes, spotting techniques and filter paper type were evaluated using a CSF-substitute of anti-JEV IgM positive serum diluted in Phosphate Buffer Solution to end-limits of detection by JEV MAC-ELISA. The optimised protocol was compared with routine, neat CSF in a pilot, retrospective study of JEV MAC-ELISA on consecutive CSF samples collected 2009-15 from three Laos hospitals.

Results: A conventional protocol, involving eluting one punch in 200µl PBS, did not detect the end-dilution, nor did multiple punches utilising various spotting techniques. However pre-cut filter paper enabled saturation with five times the volume of CSF-substitute, sufficiently improving sensitivity to detect the end-dilution. 132 CSF samples stored as dried CSF spots for one month at 25-30ºC showed 81.6% (65.7-92.3 95%CI) positive agreement, 96.8% (91.0-99.3 95%CI) negative agreement and a kappa coefficient of 0.81 (0.70-0.92 95%CI) results from neat CSF.

Conclusion: The novel design of pre-cut filter paper saturated with CSF could provide a useful tool for JEV diagnostics in settings with limited laboratory access. It has the potential to improve national JEV surveillance and inform vaccination policies. The saturation of filter paper also has significant implications for use in the wider context of pathogen detection, including dried spots for detecting other analytes in CSF, and other body fluids.
Development of a rapid point of care immuno-filtration assay for serodiagnosis of cutaneous anthrax in India

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Background: Anthrax, caused by *Bacillus anthracis* is known to occur globally since antiquity. In human, the disease manifests itself in one of three forms: cutaneous, gastrointestinal or pulmonary depending upon the route of spore entry. Cutaneous anthrax is a disease of public health importance also in country like India. Therefore, there is a need to develop an improved, simple, sensitive, specific, user-friendly, field usable, cost-effective and universal detection method for serodiagnosis of anthrax in human and animals.

Methods & Materials: Protective antigen (PA) and lethal factor (LF) genes were cloned and expressed in *E. coli*. The recombinant proteins were purified to homogeneity. A flow through system with nitrocellulose membrane was optimized by coating 1 µg of each protein and 1 µg of rabbit IgG as a control. A 30 µl serum sample was added on to membrane followed by addition of protein A conjugated colloidal gold. The appearance of color on spots was observed for the results.

Results: The detection sensitivity of the assay was found to be 10 µg/ml anti-toxin IgG. The test system was evaluated using anthrax infected sera (n=150) collected from patients presenting typical clinical symptoms of anthrax from anthrax endemic area in India. Sera from apparently healthy human (n=250), vaccinated individuals (n=5) and non-anthrax infected persons (n=30) were also tested. Flow through system was found to be 100 % sensitive and specific as compared to 93.65 and 98.44% sensitivity and specificity in ELISA.

Conclusion: The system is very rapid and takes only 2 min. The system is extremely useful for point of care diagnostic assay for clinical samples. This can be used for surveillance of anthrax infection in human as well as animals also.
Molecular biology technique combined with Fine needle aspiration cytology revealing the diagnostic dilemma in tubercular lymphadenitis cases

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Background: Extra-pulmonary Tuberculosis in the form of tubercular lymphadenitis (TBLN) accounts for 30-40% of Tuberculosis cases. Tuberculosis lymphadenitis is not always associated with the clinical symptoms like fever, cough, weight loss etc which makes its diagnosis difficult. Moreover, conventional investigations like culture and Ziehl Neelsen staining (ZN) used for providing the bacteriological evidence of TBLN have their own limitations. These cases therefore remain undiagnosed and become worse when left untreated. Such cases have potential to spread tuberculosis and thus are problem areas to the public health. We aim to reveal the diagnostic dilemma by molecular biology technique based on real-time Polymerase chain reaction (PCR) with the hypothesis that Real-time PCR performed on lymph node aspirates will be able to diagnose tubercular lymphadenitis cases which lack clinical and bacteriological evidence of tuberculosis on conventional methods.

Research problem with aproach

Methods & Materials: Cross sectional study: Fifty patients with enlarged lymph node, enrolled in this study were taken for cytological evaluation to Department of Pathology, Jawaharlal Nehru Medical College, Wardha, India. Informed consent was taken. Lymph nodes were subjected to Fine needle aspiration cytology (FNAC); aspirates obtained were used for studying morphology, ZN staining, culture and TaqMan based real-time PCR on target insertion sequence IS6110. The results were recorded and data analyzed in Software package for statistical analysis.

Inclusion and exclusion criteria and study process

Results: Female to male ratio was 1.3:1. Most common site among lymph nodes was cervical in 72%, cases. Morphologically necrotizing granuloma was seen in 20/30 cases diagnosed TBLN on Fine needle aspiration cytology, ZN staining was positive only in 15 cases, culture was positive only in 20 cases and TaqMan based real-time PCR findings were positive in 35 cases. Ten cases missed for TBLN on conventional methods (culture and ZN stain) were diagnosed by FNAC and PCR. Five cases missed for TBLN on FNAC were diagnosed by PCR. Diagnostic accuracy of real-time PCR was found to be 100%.
Conclusion: A positive real-time PCR finding on aspirates of lymph nodes reveals the diagnostic dilemma in tubercular lymphadenitis cases where there was no clinical suspicion and bacteriological proof was lacking. In addition real time PCR helps to diagnose the cases missed on morphology.
Baseline titres of Salmonella agglutinins in the healthy population in Sri Lanka
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Background: Enteric fever is endemic in Sri Lanka. The Widal test is still commonly performed for diagnosis. Due to difficulty in obtaining acute and convalescent phase sera to detect a four-fold rise in titre, a single Widal test is preferred. A significant number of healthy persons also carry antibodies to *Salmonella* Typhi and *Paratyphi A*. Therefore, it is necessary to determine the baseline *Salmonella* O, H and AH agglutinin titres in the general population to determine cut off values that denote acute infection. Titres beyond this cut off value could be regarded as significant and used for the diagnosis of enteric fever.

Methods & Materials: Five hundred and one (501) serum samples from healthy blood donors, collected from 31 blood banks across Sri Lanka in 2012/13, were tested for *Salmonella* O, H, and AH agglutinins. The tube agglutination test was used with dilutions ranging from 1:20 to 1:1280. Age and sex of study participants were recorded.

Results: Of 501 serum samples, 290 (58%) were found to be positive for any agglutinin. However, 97.5% of the positive sera had O, H, and AH agglutinin titres less than or equal to 80, 160 and 80 respectively. The rate of H and AH agglutinin positivity was significantly higher in females with a baseline titre for AH agglutinin of 160 (versus 80 in males). Test positivity rates for all agglutinins was lower in the population below 20 years but this was statistically significant only for O agglutinins. O agglutinin positivity rates remained steady after the age of 20 while H positivity rates rose with age.

Conclusion: The study shows that though the background prevalence of *Salmonella* O, H and AH agglutinins in the healthy population in Sri Lanka is high, they are found at low titres, with baseline titres of *Salmonella* Typhi O, *Salmonella* Typhi H, and *Salmonella* Paratyphi AH agglutinins being 80, 160 and 80 respectively. Therefore the Widal test can still be used as a tool for the diagnosis of enteric fever. It is important to consider sex and age when interpreting a positive test and determining cut off titres for acute infection.
Fetuin-A as a biomarker to predict invasive Pneumococcal disease in children

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**Background:** *Streptococcus pneumoniae*, a neuraminidase-producing pathogen, can cause invasive pneumococcal disease (IPD) with or without hemolytic uremic syndrome (HUS) in humans. We aimed to identify abundant serum asialoglycoproteins after pneumococcal neuraminidase treatment. We hypothesized that serum sialoglycoproteins such as fetuin-A can serve as a biomarker to predict IPD or HUS.

**Methods & Materials:** We constructed serum sialoglycoprotein profiles before and after pneumococcal neuraminidase treatment using proteomic approach. We analyzed the clinical characteristics and serum samples from 46 pediatric patients with pneumococcal infection to verify the predictive role of fetuin-A in IPD. Serum fetuin-A levels were determined by enzyme-linked immunosorbent assay.

**Results:** Fetuin A was identified after neuraminidase treatment and lectin capture. Bovine fetuin inhibited the activity of neuraminidases with IC\textsubscript{50} at 2.59 mg/mL for NanA, 2 mg/mL for NanB and 1.2 mg/mL for NanC. Mean fetuin-A levels in the HUS patients was significantly lower (207 ± 80 mg/L, \(p<0.001\)) than in patients with lobar pneumonia (610 ± 190 mg/L) as well as the healthy controls (630 ± 250 mg/L). In comparing HUS with necrotizing pneumonia and lobar pneumonia, the ROC area under the curve was 0.842; a cutoff value of 298 mg/L yielded sensitivity of 92.9% (95% CI: 68.5%–98.7%) and specificity of 71.9% (95% CI: 54.6%–84.4%).

**Conclusion:** By qualitative and quantitative analysis of serum fetuin-A in pneumococcal infections, we may identify complicated pneumonia with or without HUS caused by *S. pneumoniae*. Serial measurements of fetuin-A also has the potential to reflect patients’ response to therapy and recovery from IPD. We recommend addition of fetuin-A to the panel of biomarkers currently used for severe IPD.
Next generation sequencing targeting drug resistance conferring genes in rapid detection of Multi-drug resistant tuberculosis

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Background: Tuberculosis (TB) is one of the public health crisis caused by Mycobacterium tuberculosis complex. The emergence of multi-drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) has affected disease management. There is a need for rapid, accurate diagnosis of TB and initiation of appropriate treatment. We have performed whole genome analysis for evaluation of drug resistance conferring mutations early in the clinical isolates from patients who fail Anti-tubercular treatment (ATT).

Methods & Materials: Clinical data and sputum samples were collected from patients who were attending a tertiary care centre with risk factors for drug resistant tuberculosis. Smear microscopy, culture (LJ media) and PCR (IS6110 / TRC4 primers) were performed to identify Mycobacterium tuberculosis (MTB). Culture isolates from patient samples were subjected to whole genome sequence analysis by Next generation sequencing technique (NGS) using Miseq, Illumina platform and the corresponding drug resistant mutations were analysed using bioinformatics databases such as TB-Profiler, TBDReaM.

Results: A total of five clinical isolates were subjected to whole genome sequence analysis by NGS technique. Of which, two isolates were from patients with pulmonary TB, who were treatment failure cases (one failed Category-1 and one failed Category-2 ATT) and considered for MDR-TB were enrolled in this study. Whole genome analysis of the first possible culture isolate after treatment failure in both strains showed evidence of high level drug resistance to Rifampicin with mutations in rpoB gene at codon L511P, S531L and for Isoniazid with mutations in katG gene at codon S315G. One isolate (failed Category-1 ATT) had high confidence drug resistance conferring mutation in embB gene at codon Q497R corresponding to Ethambutol resistance. Some major drug resistant mutations to Fluoroquinolones were observed in gyrA gene (E21Q, S95T, G668D) in both genomes. Other non-synonymous substitutions were also observed in aphC gene at W53L (Isoniazid), oxyR gene at L13F (Isoniazid) and gyrB gene at M291I (Fluoroquinolones).

Conclusion: Genomic-wide analysis of drug resistance suspected isolates is recommended in order to find the possible drug resistance early in the therapy period. Whole genome analysis of drug resistance by NGS technique enables rapid identification of drug resistant tuberculosis and initiation of appropriate therapy.
Using peptidoglican associated lipoprotein of legionella pneumophila as a urinary antigen for development of an indirect sandwich ELISA

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Background: Urinary antigen testing has been proven to be the most powerful diagnostic method to detect Legionella antigen which is recognized as the causative agent of Legionnaires disease. L. pneumophila peptidoglycan-associated lipoprotein (PAL) protein is a extremely conserved antigen among Legionella species.

Methods & Materials: Rabbit and rat anti-PAL immunoglobulin G (IgG) antibodies were produced by immunization with the purified, recombinant PAL (r-PAL) protein of L. pneumophila serogroup 1 and used as capture and detection antibodies in the PAL antigen-based enzyme-linked immunosorbent assay (ELISA) to detect urinary PAL antigen. Urine samples obtained from rats experimentally infected with L. pneumophila serogroup 1. The performance of the PAL antigen-based ELISA was measured on 40 infected urine samples and 40 controls obtained from the uninfected rats.

Results: After choosing the cutoff value of 0.192, the sensitivity and specificity of the PAL antigen-based ELISA were 87.5% and 97.5%, respectively. The results obtained by PAL antigen base ELISA were compared with those obtained by Biotest. All of the control human urine samples were negative by the PAL antigen-based ELISA.

Conclusion: The present report represents an extension of our efforts to design an ELISA kit for detection of the PAL urinary antigen in Legionnaires disease (LD). The PAL antigen-based ELISA assay was relatively simple to perform, precise, highly sensitive and specific, and reproducible. The PAL antigen-based ELISA results yielding values which are almost equivalent to those found with the same samples run by the Biotest EIA. Taken together, the data indicated that the PAL antigen-based ELISA described here is the first indirect sandwich ELISA for urinary antigen detection which it could easily be applied for diagnosis of LD.
Background: Diagnostic methods are becoming a crucial component of malaria control and prevention. Improved ability to diagnose malaria may prevent many unnecessary antimalarial treatments and should also allow prompt attention to other causes of fever when malaria is ruled out.

Methods & Materials: This study compared the diagnostic accuracy of Bioline SD (HRP-2) and ACON (HRP-2/Aldolase) based RDTs with microscopy. Blood samples were collected from 200 asymptomatic and 60 symptomatic subjects in Ado-Odo/Ota LGA, Ogun state. The blood samples collected were first analyzed using the Rapid Diagnostic Tests and then stained with Giemsa stain and viewed under the microscope.

Results: Out of the samples collected from asymptomatic subjects, 44% were males while 66% were females. Among the samples from symptomatic patients, 58.3% were females. The overall incidence of falciparum malaria among the study population by microscopy was 32.3%. Among the asymptomatic patients only, the percentage incidence from microscopy was 26.5% with 11% males and 15.5% females; by Bioline SD (HRP-2) kits, the incidence was 17% with 6.5% males and 10.5% females; while by ACON (HRP-2/Aldolase), percentage incidence was 18% for *P. falciparum*, 6% were males and 12% were females. Among the symptomatic subjects, incidence rate of *P. falciparum* malaria was 42% by ACON kits, 38.3% by Bioline SD and 51.7% microscopic method with 58.1% males and 41.9% females. Two samples were positive for *P. vivax* when diagnosed with ACON (HRP-2/Pan) but was not detected by microscopy.

Conclusion: There is the need to improve on the efficacy of available Rapid Diagnostic Test methods and their sensitivity in indicating the presence of *P. falciparum* and *P. vivax* antigens in the blood.
Mild encephalitis/encephalopathy with a reversible splenial lesion caused by Legionnaires' disease presenting with cerebellar ataxia symptoms and impaired consciousness

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Background: Mild encephalitis/encephalopathy with a reversible splenial lesion (MERS) occurs due to infection or drugs. When patients with Legionnaires' disease present with articulation disorder and/or impaired consciousness, MERS should be considered as a differential diagnosis.

Methods & Materials: We report a case of MERS patient who developed Legionnaires' disease with cerebellar ataxia symptoms and impaired consciousness. The subject was 51-year-old Japanese man, who was admitted to Tokyo Takanawa hospital due to fever, articulation disorder and impaired consciousness. These symptoms started one day before the hospitalization. There was no other noteworthy medical history. Difficulty in holding a standing position was observed, and the score for finger nose test was poor. The chest auscultation confirmed the coarse crackle in the right chest. Blood tests detected high count of leukocytes, low count of platelets, hyponatremia, mild renal dysfunction, and high values for creatine kinase and C-reactive Protein. The urine sediment test showed a hematuria and proteinuria. There was no significant abnormality in the cerebrospinal fluid examination.

Results: The head computed tomography (CT) examination did not show noteworthy findings, but the head magnetic resonance imaging (MRI) examination showed high signals on T2WI and diffusion weighted imaging in the splenium corporis callosi. The X-ray and CT exams in chest detected the infiltration shadow around the lower right lung field, and the Legionella Urinary Antigen Test was found positive. From these observations, on day 2 in hospitalization, we diagnosed the patient as having MERS associated with Legionella pneumonia. Ceftriaxone, Vancomycin, and Acyclovir were administered from the time of admission, and it was changed to Levofloxacin alone after the diagnosis. On day 9, Levofloxacin was changed to Azithromycin because the drug eruption was suspected. The fever decreased over time after admission, and articulation disorder and disturbance of consciousness also disappeared. The head MRI examination on day 9 confirmed the high signals in the splenium corporis callosi disappeared, and the subject discharged on day 14.

Conclusion: MERS associated with Legionnaires’ disease may be more common than previously considered since head MRI examination is not necessarily performed. MERS should be considered when patients with Legionnaire’s disease present with articulation disorder and/or impaired consciousness.
Field evaluation of a novel loop mediated isothermal amplification (LAMP) assay for molecular diagnosis of asymptomatic malaria in a field setting in sub-Saharan Africa

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Background: Parasitological confirmation of malaria prior to treatment is recommended by the World Health Organisation (WHO). However, more sensitive and high throughput diagnostic tools are required to support the new pursuit for malaria elimination. The challenge of deploying molecular tools like polymerase chain reaction (PCR) in peripheral settings where they are most needed remains a concern, thus isothermal amplification methods such as loop mediated isothermal amplification (LAMP) are being developed. In this study, we report the evaluation of a novel, in-house developed LAMP assay targeting the apicoplast genome, in a field setting in sub-Saharan Africa.

Methods & Materials: The study was carried out in the screening stage of an ongoing trial (PRINOGAM) comparing different single doses of Primaquine on gametocyte carriage among individuals with asymptomatic malaria. Samples were collected from consenting individuals in the study villages around the Medical Research Council (MRC) field site in Basse, The Gambia, from October to December 2014. From a single finger prick, samples were collected from 341 participants for microscopy, RDT and dried blood spots (DBS). DNA was extracted from the DBS using a simple methanol extraction method for the LAMP assay, and a QIAamp DNA mini kit for the reference PCR assay.

Results: A mean of 27 ± 9.5 samples were collected daily. Median age of individuals screened was nine years, ranging from 1-68 years; most study subjects (78%) were less than 15 years old. Malaria prevalence by microscopy was 30% (104/341) and although prevalence by RDT (126/341; 37%) and LAMP (127/341; 37%) did not differ, the agreement was significantly different. Compared to the reference PCR method, LAMP had a sensitivity of 92%, specificity of 97%, positive predictive value (PPV) and negative predictive value (NPV) of 95%. Microscopy had a sensitivity of 78%, specificity of 99%, PPV of 98% and NPV of 88%. Sensitivity, specificity, PPV and NPV for RDT were 76, 88, 79 and 86%, respectively. The turnaround time for the LAMP assay was approximately 3 hrs 30 mins.

Conclusion: As it becomes more feasible to deploy molecular tools for diagnosis of malaria at peripheral levels, global eradication of malaria can gradually become a reality.
Diagnosis of Iranian MSMD patients in a proliferation and cytokine production setting

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Background: Mendelian susceptibility to mycobacterial diseases (MSMD) is a rare inheritance syndrome, characterized by a disseminated infection in children following BCG vaccination performed at their birth time. In the infected children with MSMD, there is a susceptibility to systemic infection with mycobacterium tuberculosis and nontuberculous including Bacillus Calmette-Guerin (BCG) vaccine

Aime:
We aimed to diagnosis MSMD patients over a period of 2 years at the main referral center for immunological disorders in Iran.

Methods & Materials: In this study, suspected patients with MSMD referred to "Immunology, Asthma and Allergy Research Institute" are studied genetically. The patients were affected with localized disseminated and recurrent lymphadenopathy after BCG vaccination at the birth time, and have normal immune system result tests. Their LTT function is normal in the exposure with PHA, is defective in the exposure to BCG. In this study, we measured IL-12, IFN gama levels to help identify patients.

Results: In this study, we have 4 controls and 8 patients that had impaired response to IL-12. Although there was no significant relationship between LTT with PHA, it becomes significance when we added BCG alive. In addition, the arrays of IL-12 (0.003), IFN gama (0.015) between controls and patient groups was significance.

Conclusion: Evaluating IFN-g and IL-12 assay can help for quick and short time diagnosis of MSMD disease (defect in IL12r1, receptor1, and defect in IL12 and IFN-g receptor). However genetic investigation in this disease is more complete and definitive for the diagnosis in these patients.
Comparative evaluation of Xpert® Carba-R assay with conventional methods for detection of carbapenemase producing enterobacteriaceae

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**Background:** Carbapenemase producing Enterobacteriaceae (CPE) are a major public health threat with high transmissibility and limited treatment options. Xpert Carba-R® (Cepheid®) is a simple PCR-based assay for rapid (<1 hour) detection of bacteria carrying carbapenem-resistance genes (KPC, NDM, VIM, OXA-48, IMP-1). The aim of this study was to assess and compare Xpert® Carba-R assay with phenotypic methods using rectal swab specimens.

**Methods & Materials:** Inclusion criteria for collection of paired rectal swabs consisted of 72 patients already proven to have CPE in abscess/blood/sputum/urine/bronchial alveolar lavage samples and 83 patients considered to be having high risk factors for CRE carriage. Methods included two swabs, one of which was initially inoculated on MacConkey Plate with a 10ug meropenem disk and then inoculated in MacConkey broth containing 10µg meropenem disk. The other swab was processed as per instructions for Xpert Carba-R (Cepheid). A phenotypic resistance was confirmed using the CLSI guidelines (M100-S23). In case of swabs with culture positive and Xpert® negative results, isolates were analyzed again by Xpert® Carba-R.

**Results:** Out of the 155 swabs analyzed, a total of 109 (70.3%) and 102 (65.8%) patients were positive by phenotypic analysis and GeneXpert respectively.

115 isolated carbapenem resistant organisms from 109 patients comprised of *E.coli* (n=59), *Klebsiellae spps.* (n=41) and *Enterobacter spps.* (n=15).

New Delhi metallo-beta-lactamase (NDM) genes were isolated in 86 patients. Verona integron-mediated metallo-beta-lactamase (VIM) genes were isolated from 2 *E.coli* and 1 *K.pneumoniae.*

Co-production of NDM and VIM genes were seen in 9 isolates. Co-production of *Klebsiella pneumoniae* carbapenemase (KPC) and NDM genes were seen in 2 *K.pneumoniae.* Imipenemase metallo-β-lactamase (IMP) were also seen co-produced along with NDM genes in an *E.coli* & *E.aerogenes* isolate.

11 swabs were CPE negative by Xpert® and phenotypically positive, 3 of 11 isolates were positive by Xpert® Carba-R.

The sensitivity, specificity, PPV, NPV of Xpert® Carba-R test was 93.5%, 100%, 100% and 86.7% respectively.

**Conclusion:** Xpert Carba-R Assay could be utilized for screening of high risk patients and implementation of infection control measures which will help in preventing spread of CPE.
Identification of B cell epitopes of in vivo expressed RD proteins in pulmonary tuberculosis (PTB) patients
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Background: Despite the newer advances in the field of TB diagnostics, there is still a need to develop a rapid,
easy and specific diagnostic test which can be used in the primary health care settings. ELISA based assays
satisfy these criteria, but due to the lack of appropriate and specific biomarkers, no specific ELISA based assay
has been designed for the diagnosis of TB. Earlier we identified some specific region of difference (RD) genes
that were up regulated in the sputum samples of PTB patients but not in disease controls by mycobacterial
whole genome microarray analysis. As, proteins encoded by these genes could be highly TB specific biomarkers
that are expressed during disease, therefore present study was designed to confirm their in vivo expression as
well as to identify the B cell epitopes of these proteins.

Methods & Materials: Peptide arrays were used to confirm the expression of upregulated genes in sputum at
protein level. Briefly peptide array slides consisting of overlapping peptides of the 5 upregulated RD proteins
were synthesized commercially and were screened using the sera of 7 PTB patients and 7 healthy controls to
look for the presence of antibodies against these proteins and to further select the specific epitopes of these
proteins which can further be used in an ELISA based diagnostic assay.

Results: Antibodies were present in the sera of PTB patients against 4 out of 5 RD proteins which further
confirm their expression in vivo in human subjects. Therefore these proteins may serve as effective and specific
diagnostic candidates in an ELISA based assay for tuberculosis. Further epitope mapping of these proteins lead
to identification of 11 peptides which were significantly recognized by all 7 (100%) PTB patients and 8 peptides
which were recognized by 6 (≥85%) of PTB patients but not in healthy controls.

Conclusion: 4 RD proteins which are highly specific to mycobacteria and absent in vaccine strain BCG have
shown expression in vivo at gene and protein level and their peptides are found to be highly immunodominant
and can further be used in an ELISA based assay for PTB.
Role of fine needle aspiration cytology in the diagnosis of cutaneous leishmaniasis

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Background: Iran is an endemic area of Cutaneous leishmaniasis. Scraping method is widely used conventionally. Fine needle aspiration (FNA) cytology is now getting more attention.

Methods & Materials: Both methods were performed on the clinically suspected cases in our study. Smears were stained using Giemsa. We compared the sensitivity, specificity and some other aspects of these two methods.

Results: Of our 400 patients, 346 had specimens that were positive for leishman body, and of these 328 were detected using both methods. However, 42 cases were confirmed positive by FNA cytology and 18 as a result of scraping smears. There was a significant difference between the two methods in the detection of leishman body and microgranuloma, slide background and patient comfort. The sensitivity of FNA cytology was greater even though the specificity was the same.

Conclusion: Our study confirmed the advantages of FNA cytology as a reliable method for the diagnosis of cutaneous leishmaniasis.
SLA-ELISA: A comparison with DAT & rK39 ELISA for identification of seroconverters

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Background: The most important drawbacks in visceral leishmaniasis (VL) population studies are the difficulty of diagnosing asymptomatic carriers. The aim of this study, conducted in a rural area of Muzaffarpur District Bihar, India was to evaluate the performance of serology to identify asymptomatic VL infection in participants selected from a TMRC cohort area.

Methods & Materials: Blood samples on filter paper were collected from 437 (seroconverters and controls) cohort participants. Samples were tested by ELISA using two different antigens: Soluble Leishmania antigens (SLA), and rK39 recombinant protein and DAT (Direct agglutination Test).

Results: When SLA ELISA compared with both rK39 ELISA and DAT showed SLA ELISA was positive in all subjects who were positive with both DAT & rk39 ELISA. SLA ELISA also detects infection in those who are negative with both tests DAT and rk39 ELISA.

Conclusion: For the diagnosis of asymptomatic infection there is no need to run both serological (DAT and rK39 ELISA) test. We also found seropositive samples from the control group might be we missed some seropositive samples with these serological (rK39 ELISA and DAT) tests so SLA ELISA could be used as marker of Infection.
Determination of absolute accuracy of diagnostic tests using Bayesian LCMs: A re-evaluation of diagnostic tests for Adenovirus

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**Background:** Viral culture is considered to be the gold standard method to identify adenoviruses from clinical samples. It can be affected by various factors like transport conditions, pH. As a consequence of this, the accuracy of the different diagnostic tests might have been inaccurately estimated. Objective of this study is evaluating the absolute accuracy of three tests using Bayesian latent class models.

**Methods & Materials:** The set of data analyzed in this study was from our prospective study. In brief total of 62 suspected viral conjunctivitis patients were recruited from Nov2013 to Jan2015, conjunctival swab was collected and processed for conventional viral culture (HEp2 cell line), IFA using pan adenoviral primary antibody (monoclonal antibody directed against hexon protein) & FITC labeled secondary antibody and Universal semi-nested PCR.

**Results:** Accuracy of each diagnosis test was evaluated, comparing with viral culture which was considered the gold standard (100% sensitivity & specificity). Overall isolation rate of adenovirus was 54.8% (34/62). Viral culture was positive in 23 patients (67.6%); PCR & IFA detected adenoviruses in 24 (70.5%) and 21 (61.7%) cases respectively. Sensitivity and Specificity of IFA & PCR was 60.9% & 82.1% and 69.6% & 79.5% respectively. The absolute accuracy of each diagnostic test was evaluated using Bayesian LCMs, which do not assume any diagnostic test as a perfect test. Analysis carried out by models provided at this web address (http://mice.tropmedres.ac) using three tests in one population (Walter & Irwig model) simplified interfaces. After analysis using Bayesian LCM, the actual sensitivity and specificity of the viral culture were reduced to 72.2% & 90.8%. The sensitivity & specificity of PCR were increased from 69.6 to 77.8% and 79.5% to 92.4%. In both ways PCR appears to be superior to viral culture in identifying adenovirus. PCR positivity in culture negative samples may be because PCR can detect even few copies of nucleic acid and non-viable particles. Also, sensitivity & specificity of IFA has increased from 60.9% to 67.6% and 82.1% to 92.9% respectively.

**Conclusion:** Our study shows that when reference test is imperfect, the accuracy of sensitivity and specificity of alternative test can be very well determined by Bayesian LCM statistical analysis.
PCR-RFLP for identification of Mucorales from clinical specimens

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Background: Mucormycosis is an emerging fungal infection associated with high mortality. The standard approaches available for the diagnosis of this condition are limited as this agent could only be isolated in 50% of samples processed due to delicate nature of the agent. Early diagnosis and institution of appropriate antifungal agents is the cornerstone of successful outcome of mucormycosis. Hence the present study was conducted to evaluate PCR-RFLP based molecular technique for diagnosis and identification of etiological agents of mucormycosis.

Methods & Materials: Fifty proven or probable cases of mucormycosis were included in the study. Conventional identification was done by microscopy and culture. Molecular diagnosis was done by amplifying the ribosomal DNA using semi-nested Mucorales specific primers of 18s region. We also standardized and evaluated the utility of PCR-RFLP of 18s region of rDNA in identifying the Mucorales. The amplified products were sequenced and were compared to evaluate the efficacy of PCR-RFLP to identify the Mucorales.

Results: All the samples showed aseptate hyphae on KOH examination. Isolation was possible in 24(48%) samples. Mucorales specific PCR targeting 18s region was 100% sensitive and the sequence revealed the species identity in all of them (R. oryzae-80%; R. microspores-10%; A. elegans-6%; and R. homothallicus-4%). The molecular pattern obtained by PCR-RFLP could only differentiate the agent up to genus level. Considering Mucorales specific PCR as standard the sensitivity, specificity, positive and negative predictive values of culture isolation (R. oryzae) was 46% (CI-30.7-62.6), 100% (CI 66.4-100), 100%, and 29% respectively. Molecular technique could correctly diagnose an additional 26(42%) cases, which were negative by culture.

Conclusion: Early rapid diagnosis is the cornerstone in the management of mucormycosis. The present study reveals the use of Mucorales specific PCR for diagnosis and PCR-RFLP for the identification of the causative agent in setup without sequencing facility.
Economic evaluation of influenza vaccine intervention

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**Background:** The objective of this study is to evaluate the economic impact of vaccine-based interventions in response to an influenza pandemic, for the city of Chicago, and compare the cost-benefit metrics between a dynamic agent-based network model and a static Markov model. Efficient allocation of clinical resources, including vaccination, minimizes the costs of deaths, hospitalizations, and outpatient visits during an influenza pandemic.

**Methods & Materials:** The social contact network is a co-location based synthetic network, generated for the city of Chicago. The transmission dynamics of the influenza-like-illness in the population is simulated using the susceptible-exposed-infectious-recovered epidemiological model. We compare the costs and benefits of different vaccine-based interventions in control and prevention of an influenza pandemic.

**Results:** We simulated a base case scenario of no vaccine intervention for the city of Chicago with a basic reproductive number of 1.5 for a resultant attack rate of 58.1% and health care cost per capita of $1,124. Applying the vaccine intervention with efficacy of 40% and compliance rate of 40% was a cost saving intervention for both the dynamic agent-based network model and the static Markov model. The net return per capita at 21$ per vaccine is $363 and $261 for the dynamic and static models respectively.

**Conclusion:** We infer that higher number of cases of Influenza are averted in the dynamic agent-based network model in comparison to the static Markov model, as well as the vaccine-based interventions are comparatively more cost effective for all age and risk groups in the dynamic model.
Attitude toward quoting research evidence in technical guidelines on the Nigeria HIV/AIDS program

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Background: In 2013 Nigeria was home to 9% of PLHIV, 10% of new infections and 14% of AIDS related
deaths. HIV programs are directed by relevant evidence based National guidelines; providers in Nigeria have
however continued to use various guidelines for patient management, stating the availability of evidence based
research from various sources in other guidelines.

Methods & Materials: We sought to review the Nigerian National guidelines for availability of references to
determine the number and characteristics of research references cited. This was intended to give a picture of
the access to, the demand and value attached to published research and evidence base among professionals in
this field at both policy and implementation level. We conducted a comparative study of 17 national guideline to
the PEPFAR 2012 Guidance for OVC. We collected data on the presence, frequency, nature, origin, level of
evidence and recentness of research referenced in the guidelines. We also analysed the average distribution of
references per size of documents; and the ratio of quoted research references to none-research references.

Results: In the 17 National guidelines reviewed, a total of 2685 pages of HIV information (mean= 158 pages
per document) was reviewed. Only 3 (18%) had a reference section; while 14 (82%) were published without a
reference section. A sum of 115 references were quoted; 42 (37%) of which was research evidence, with one of
the guidelines being responsible for 71% of all the research evidence quoted. The average number of
references per page in the 17 National guidelines was 60 times less than was obtainable in a single PEPFAR
guideline. The average ratio of none-research to research references quoted was 1:0.5 in the National
guidelines as opposed to 1:17 in the PEPFAR guidelines.

Conclusion: There is a limited access to quoted research evidence in Nigeria National HIV program guidelines.
The lack of reference in National guidelines may cast doubt on quality of evidence therein and question the
continued use of non-local evidence to direct interventions.
Equipping India’s community health worker supervisors with a mobile phone based supervisory application
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**Background:** India has a network of over 825,000 women who serve as Accredited Social Health Activists (ASHA) in rural communities. Until recently this cadre of community health workers lacked a supportive supervision structure and system. A 2011 evaluation of the program identified incomplete training, limited management and monitoring structures as major barriers to optimizing ASHA’s effectiveness in delivering services at community level. CRS India in 2012 piloted a mHealth application for project supported supervisors in Kaushambi district in India that provided real time information to guide supportive supervision of ASHAs.

**Methods & Materials:** From September 2012 until September 2014, the supervisory application was implemented in one block of the district and data on supportive supervision and lessons learnt were shared with the government. In December 2013 Government of Utter Pradesh decided to roll out a new cadre of community health workers, ASHA Sangini’s to provide supportive supervision to ASHAs. CRS worked closely with the government to develop a mobile phone based application designed as a tool to improve ASHAs performance.

**Results:** Between September 2012 and September 2014, 16 quality parameters on engagement of ASHAs with clients have shown consistent improvement. The percentage of low coverage ASHAs has reduced from 55% to 15 %, and average number of home visits during the antenatal period has almost doubled from 1.18 to 1.95. The application helps collecting functionality data on 10 key performance indicators which is used to provide structured feedback for improvement. Currently the application is helping 139 ASHA Sanginies to keep track of the performance of 2670 ASHAs.

**Conclusion:** Technology can be used to strengthen supportive supervision by providing real-time data on ASHA performance that Sanginis use to provide individualized feedback and guidance for improvement. It seems to have potential for further scale up and replication.
An economic evaluation of a livestock anthrax vaccination program in high-risk regions of the country of Georgia

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**Background:** In 1995, legislation was passed requiring the prevention of epizootic diseases in Georgia through mandatory livestock anthrax vaccinations and other mechanisms. In 2007, responsibility for prophylactic livestock vaccinations shifted from the government to livestock owners. Between 2010 and 2012 there was a substantial reduction in anthrax vaccinations, a three-fold increase in livestock cases and a four-fold increase in human cases. The government resumed responsibility in 2013. We analyzed the economic feasibility of a government-funded livestock anthrax vaccination program in the predominately affected regions of Kvemo Kartli and Kakheti.

**Methods & Materials:** We used an Excel model to analyze program costs (livestock vaccination administration) and benefits (averted livestock and human cases) from a government perspective. Cost per human case averted was the primary outcome. Based on a prior study, we assumed a 1:1 ratio for animal to human cases, and calculated a minimum correction factor of 3.8 to account for livestock case underreporting. We performed sensitivity analyses to determine how changes to model inputs affected results. Sensitivity analyses were guided by subject matter expert opinion.

**Results:** We estimated first-year vaccination costs, at 100% coverage, in high-risk regions was 194,451 Georgian Lari (GEL)/$86,743 US. This translated to 0.46 GEL/$0.21 US per animal vaccinated (assuming 2 vaccines/animal in year 1). Vaccine, salary and transportation accounted for 80% of program costs. Accounting for underreporting, 46 livestock cases and 46 human cases would be averted. The cost per human case averted was 4,227 GEL/$1,902 US. Simultaneously decreasing vaccine cost by 40% (from 0.08 GEL/$0.05 US to 0.05 GEL/$0.03 US), and increasing anthrax prevalence by 150% (from 0.011% to 0.027%) changed the cost per human case to 1,431 GEL/$644 US.

**Conclusion:** These data can aid the government of Georgia in decision-making regarding continued funding of livestock anthrax vaccination programs.
Ending the neglect: The role of global policy advocacy in addressing Neglected Tropical Diseases (NTDs)
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Background: Global and regional economic and political platforms like the Group of 7 (G7) and the Association of Southeast Asian Nations (ASEAN) and development banks like the Inter-American Development Bank (IDB) and the World Bank, are increasingly engaged in health and development agenda-setting and policy guidance. Global economic and political platforms facilitate innovative, collaborative partnerships and development banks offer opportunities to pool resources, knowledge and influence to demonstrate greater impact, thereby building support and evidence for these models to be replicated. The Global Network for Neglected Tropical Diseases (The Global Network) has engaged with such platforms to push for progress on controlling and eliminating neglected tropical diseases (NTDs).

Methods & Materials: NTDs disproportionately affect the world’s most socially and economically marginalized populations, furthering the cycle of poverty and poor health outcomes. The most common NTDs, those that affect 1.4 billion people worldwide, are easily and inexpensively prevented and treated through annual or biannual mass drug administration. In many cases the drugs are donated and administration of medication can easily be integrated into existing health systems. These major barriers have been overcome, but more can be done to accelerate progress toward WHO’s 2020 NTD control and elimination targets.

Results: Increased energy, political commitment and resources, and encouraging cross-sectoral and cross-country collaboration through global policy makers can help to accelerate the progress of existing country programs, scale the successes across geographic areas, and mobilize resources.

Conclusion: This presentation will provide examples of global advocacy and highlight tools that have been helpful in convincing policy makers to take an interest in NTDs, focusing on increased resource mobilization and opportunities to leverage existing programs and resources to fund pilot programs and document innovative models. Given the entry of new platforms and funding entities with an interest in health and development, like the BRICS and the New Development Bank, a review the global policy advocacy strategies and opportunities is particularly timely.
Potential impact and economic value of dengue vaccination in 10 endemic countries
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Background: With about 100 million apparent infections occurring annually, dengue is a major international public health concern. To date, no specific treatment is available for this disease but the Sanofi Pasteur vaccine candidate has, in 2014, successfully demonstrated its protective efficacy against symptomatic dengue in two Phase 3 efficacy trials performed in five Asian countries (Indonesia, Malaysia, Philippines, Thailand, Vietnam) and 5 Latin American countries (Brazil, Colombia, Honduras, Mexico, Puerto Rico).

Methods & Materials: Results observed during the phase 3 efficacy trials were used to fit an age-structured, host-vector, and serotype-specific compartmental model, combined with country-specific information on the cost of dengue illness, dengue routine surveillance data, and population demographics. This model allowed assessing the potential public health and economic impact of various vaccination strategies for the 10 countries in these phase 3 trials. The strategies analyzed included routine vaccination alone (9 year-olds annually) and routine vaccination combined with alternative catch-up campaigns (targeting from 2 to 20 age cohorts and completed in 2, 3 or 4 years). The economic value of vaccination programs was assessed from a societal perspective using WHO criteria for cost-effectiveness, including offsets for medical costs averted. All costs were expressed in 2015 US$.

Results: Results indicated that 20% to 30% of dengue cases can be prevented at the population level by using routine vaccination alone. The combination of routine vaccination and the broadest catch-up campaigns could reduce the number of dengue cases in the population over 10 years by 70%. The threshold price per dose for which vaccination can be considered as cost-effective ranged from US$20 to US$100 according to country and vaccination strategy considered, or US$60 to US$300 for the 3 recommended doses.

Conclusion: The analysis indicates that the implementation of dengue vaccination programs has the potential to be cost-effective in all 10 endemic countries in the Phase 3 efficacy trials.
Improving HIV service delivery in detention centers and ART facility in Odessa, Ukraine

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Background: Under USAID funded RESPOND project, a collaborative Quality Improvement (QI) effort was initiated in March 2013 with two detention centers and an Anti-retroviral treatment (ART) site in Odessa; their respective aims were to improve the HIV continuum of care among the rural population and to increase the coverage of detainees with HIV Testing and Counseling (HTC) services and enrolment in care. Before the improvement effort, in 2012, only 3.0% of detainees received HIV counseling, of which 19.2% were tested for HIV. Furthermore, there was no reliable mechanism to link released HIV-positive detainees with ART services.

Methods & Materials: The three sites applied a Plan-Do-Study-Act QI Model to test changes; this model included teamwork and coordination with the Odessa AIDS Center; monthly self-monitoring against improvement indicators, QI coaching visits, and sharing results during quarterly learning sessions. After 12 months of implementation, a data review and qualitative assessment survey were conducted.

Results: Over one year, the two detention centers increased the coverage of HIV counseling services for detainees from 44% to nearly 70%. The coverage of HIV testing increased on average from 32% to 70%. The percentage of detainees that tested positive who were enrolled in care with the AIDS Center increased from 0% to 40% after introduction of health system changes.

The ART Center increased the proportion of HIV patients enrolled into care within 30 days after the confirmatory HIV test from 55% to 70%. The percentage of patients that underwent regular checkups increased from 42% (June 2013-October 2013) to 80%.

Conclusion: The key results of the project were the following: for the Odessa ART Center - 1) increased timely enrolment of HIV positive individuals in care, and 2) improved attendance of HIV positive patients at regular check-ups; and 3) increased coverage with HTC services. These results indicated that more people were diagnosed with HIV at an earlier stage and more of them were enrolled in care in a timely manner and benefitted from the known efficacy of ART.
Policy options for state-based PCV rollout in India: The evidence base

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Background: One in five child deaths occur in India; pneumonia and meningitis are major causes. Among Indian children aged 1-59 months, more than 160,000 deaths were attributed to pneumonia or meningitis in 2015. Pneumococcus is the most common cause of bacterial pneumonia, responsible for one-third of child pneumonia deaths. It is vaccine-preventable and more than 130 countries have introduced PCV into their national immunization programs. In these countries, routine PCV use has virtually eliminated vaccine-serotype pneumococcal pneumonia and invasive pneumococcal disease, and reduced severe pneumonia and all-cause mortality. However, PCV is not currently part of India’s Universal Immunisation Programme (UIP). The relative health need and rollout feasibility in each of Indian state was analyzed to develop policy options for rollout.

Methods & Materials: This analysis considered indicators of disease burden and mortality, access to and equity of care, governance, vaccine coverage, rollout capacity, and surveillance capacity. Using available data, these indicators may be assessed and ranked in identifying which states to include in the initial cohort.

Results: Policy options were identified based on the relative ranking of each state on need and immediate feasibility. Under each selection strategy—need only, immediate feasibility only, need and feasibility, and surveillance capacity—we assumed an initial rollout of 20% of the birth cohort. We applied DTP3 coverage to state birth cohorts for an estimate on coverage under rollout scenarios. Under a need-based selection process, fewer states would be able to introduce because of large size of the high need state populations. Conversely, under a feasibility-based process the rollout cohort could include 15-20 states; states with high immediate feasibility are significantly smaller.

Conclusion: Introducing PCV into India’s UIP has the potential to dramatically impact child morbidity and mortality. Decisions around resource allocation depend on India’s priorities and needs; analyzing state-level need and feasibility can inform state selection through a structured data approach. Using available data to rank states on key indicators allows for evidence-based policy options to identify states for the initial rollout cohort. In selecting states for initial phases of a potential PCV introduction, the Government of India may use all or part of these options.
Case management of childhood diarrhoea in low-and-middle-income countries: Time trends and country-wise changes during 1985-2012
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Background: Diarrhoea case management indicators obtained from national-level health surveys may be indicative of the impact of diarrhoea control programs. We aimed to assess the time trends and country-wise changes in the diarrhea case management indicators among under-five children in low-and-middle-income countries.

Methods & Materials: We analyzed aggregated data from Demographic and Health Surveys and Multiple Indicator Cluster Surveys done between 1986 to 2012. Two-week prevalence rates of diarrhea, proportion of children who were taken to healthcare provider, proportions of children who were given oral rehydration solution (ORS) alone, ORS or recommended home fluids (RHF) and 'increased fluid intake' were analyzed. We estimated the total number of children who had suffered from diarrhoea, and those who were given recommended treatment. Overall time trends across countries using country-level panel data and change in indicators between two sequential surveys were assessed.

Results: Overall, yearly increase in case management indicators was 1.3 - 2.5%. In 2012, <50% of children were given correct treatment for diarrhea. Annually, an estimated 300 to 350 million children were not given oral rehydration therapy or taken to a health care provider. Overall, care seeking for diarrhea, increased between pre-2000 to post-2000 periods (34.8% to 45.4%); whereas ORS use rates increased from 28.1% to 35.2% and use of ORS/RHS increased 35.7% to 41.5% but the rate of 'increased fluid intake' decreased by 41% to 26.1%. Country-level trends showed that proportion of children taken to healthcare provider, ORS and ORS-RHF use rates had increased in 34, 47 and 37 countries respectively. However, seeking care from a healthcare provider, ORS/RHS and ORS use rates had increased by ≥10% in about 20 countries only and in 30 countries these indicators had increased by <10% . In 28 countries, the rate of 'increased fluid intake' had decreased and in 20 countries it decreased by ≥10% .
Graph-2 country-wise changes

**Conclusion:** Overall very slow and a limited progress has been made in diarrhea case management. Very few countries have reached the desired targets. A better understanding of care seeking behaviors of the care givers and case management practices of health care providers is needed to improve diarrhea case management.
Adverse reactions to field vaccination against lumpy skin disease in cattle

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Background: Lumpy skin disease (LSD) is an emerging pox disease that can cause serious losses in cattle industry due to decreased productivity, cost of veterinary treatments, death, and impact on the international trade of live animals and animal products. The disease originated from Africa, but it has spread to countries of the Middle East and poses a serious threat to Europe and Asia. Recently, field veterinarians in Jordan reported a range of clinical signs seen after the LSD vaccination in cattle.

Methods & Materials: During the outbreak of LSD in Jordan, farmers outside the outbreak governorate (Irbid) were recommended to vaccinate their cattle of all ages, types and sexes using a sheep pox virus (SPPV) RM65 vaccine, Jovivac. After the vaccination campaign was initiated, post vaccinal reactions were suspected. Affected farms were investigated and data collected about animals on each farm that practiced vaccination against LSD.

Results: Sixty-three dairy cattle farms, with a total of 19,539 animals, were included in the study. Of those, 56 farms reported adverse clinical signs after vaccine administration. The duration between vaccine administration and appearance of adverse clinical signs ranged from 1 to 20 days (Mean = 10.3, SD 3.9). Clinical signs were similar to those observed with natural cases of lumpy skin disease. These included fever and variable sized cutaneous nodules that could be seen anywhere on the body. Some cattle had swollen lymph nodes, while a few pregnant animals aborted. The percentage of affected cattle ranged from 0.3 to 25% (Mean = 8, SD 5.1). Fever, decreased feed intake, and decreased milk production were seen in 83.9, 85.7, and 94.6% in cattle on the affected farms, respectively. All affected cattle displayed skin nodules over their entire bodies. No mortalities were reported due to vaccine adverse reactions. Duration (course) of clinical signs ranged from 3 to 20 days (Mean = 13.7, SD 4.1).

Conclusion: LSD vaccines can be associated with severe reaction that can be confused with natural infection. Further studies are warranted to identify safe vaccines for this disease.
Maximizing detection of dengue virus serotypes by a modified reverse transcription-polymerase chain reaction assay in India: presence of co-infection with multiple serotypes

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**Background:** Dengue surveillance uses reverse transcription-polymerase chain reaction (RT-PCR), although no standardized method for Indian serotypes exists. We compared efficacies of known and modified primer sets targeting envelope (Env) and capsid pre-membrane (C-prM) genes for detection of circulating dengue virus (DENV) serotypes in southern India.

**Methods & Materials:** Acute samples from children with clinically-diagnosed dengue were tested for NS1/anti-dengue-IgM. Viral RNA was extracted and two-step nested RT-PCR was done using 3 methods; in the first, consensus primers targeted 654bp of C-prM (CprM654) with a modified reverse primer; the second targeted 511bp of C-prM (CprM511), and the third targeted 641bp of Env (Env641). To ensure accuracy, sequencing (ABI, Applied Biosystems) was done on all RT-PCR-positive samples; DENV sequences were aligned using ClustalW, and compared with NCBI's GenBank database.

**Results:** Among 162 children (mean age 6.9yrs±4.3; males 61.0%) hospitalized between Nov 2014-Mar 2015, 113 were 'dengue-positive' (111 NS1-positive and 2 dengue-IgM-positive), and 49 were 'negative' (undetectable NS1/dengue IgM/IgG). Among 113 positives, PCR detected 84 (74.3%) by CprM654, 66 (58.4%) by CprM511, and 72 (63.7%) by Env641, suggesting high suitability of CprM654 for regional DENV serotyping. Among 49 'negative' samples, 10 (20.4%) were detected by CprM654, 11 (22.5%) by CprM511, and 11 (22.5%) by Env641. Overall detection rate using all three methods sequentially was 81.4% (92/113) among positive and 38.7% (19/49) among negative samples. Consensus serotype distribution (including multiple-serotype co-infection) was DENV-1 (66, 59.4%), DENV-2 (28, 25.2%), DENV-3 (19, 17.1%) and DENV-4 (1, 0.9%). Co-infections with multiple serotypes were seen in 8 children (4.9%); among these, increased severity and death was seen in one child, and moderate severity in 4 children. Discordant results were seen in 6 (3.7%) samples, signifying either varying sensitivities among RT-PCR methods, or presence of recombinant virus.

**Conclusion:** This is the first sequencing-confirmed optimization study showing improved detection of circulating DENV serotypes from symptomatic Indian children, using a modified RT-PCR method and a testing algorithm. Our results showed a significant rate of co-infection with multiple serotypes. This method may be used with immediate effect for national dengue surveillance, which is critical in hyper-endemic countries like India, and for future vaccine studies.
The role of diabetes in the severity of 2009 influenza A (H1N1) and the Middle East respiratory syndrome coronavirus (MERS-CoV): A systematic review and meta-analysis

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Background: A number of acute respiratory infections outbreaks such as the 2009 influenza A (H1N1) and the Middle East respiratory syndrome coronavirus (MERS-CoV) have emerged and presented a considerable global public health threat. Epidemiologic evidence suggest that diabetic subjects are more susceptible to these conditions. However, the global influence of diabetes to the severity of H1N1 and MERS-CoV is yet to be evaluated. Objective: The aim of this study was to carry out a systematic review and meta-analysis documenting the prevalence of diabetes in severe H1N1 and MERS-CoV to enable estimating its contribution to the severity of these conditions.

Methods & Materials: A search strategy was developed for online databases (PubMed, Ovid MEDLINE, Embase and Embase Classic) using H1N1, MERS-CoV and DIABETES as search terms. Reports documenting the prevalence of diabetes in these conditions were identified. Meta-analysis for the proportions of diabetes in severe conditions (95% confidence intervals, CI) was carried out (29 H1N1 studies, n=92,948 subjects and 9 MERS-CoV studies, n=308). Weighted averages of the extracted information and subgroup analysis (by region) were carried out.

Results: Average age of H1N1 patients (38.0±9.2 yrs) was lower than that MERS-CoV patients (54.9±10.1 yrs, p<0.05). The prevalence rates of clinical symptoms such as pyrexia, dyspnea, pharyngitis and pertussis were comparable between the two conditions. Compared to MERS-CoV patients, H1N1 subjects exhibited 3-fold lower prevalence of cardiovascular diseases and 2- and 4-fold higher obesity and immunosuppression rates, respectively. The prevalence of diabetes in severe H1N1 was 14.6% (95%CI: 12.3-17.0%; p<0.001), a 3.7-fold lower than in MERS-CoV (54.4%; 95%CI: 29.4-79.5; p<0.001). The contribution of diabetes to the severity of H1N1 from Asia (18%) and North America (20%) was 2-fold higher than that from South America (9.8%) and Europe (10%).

Conclusion: The effect of diabetes is 4-fold higher in MERS-CoV than in H1N1 and may play a significant role in the susceptibility to these conditions and vulnerability to their ensuing severe complications. The high prevalence of diabetes in H1N1 in North America and Asia may reflect its elevated prevalence in these regions.
Concurrent dengue and malaria coinfection: Observations from a central Mumbai hospital

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**Background:** Coinfection by Dengue and Malaria is not uncommon, though studies are scarce. Both remain among the major causes of acute febrile illnesses in India. The objective of this study is to understand and observe the interplay of Dengue and Malaria, compare their clinical and laboratory features, and analyze the outcomes.

**Methods & Materials:** A comparative, retrospective study of Dengue, Malaria, and their coinfections was conducted during 2 consecutive monsoons (June-November 2014 and 2015) in a Mumbai hospital. Febrile patients during this period were investigated for both Dengue and Malaria simultaneously. Elisa (NS-1/IgM) and peripheral smear examination was done to confirm Dengue and Malaria, respectively. Clinical comparison of signs and symptoms, severity, and outcomes, as per predefined criteria, was systematically carried out. Relevant laboratory parameters were compared.

**Results:** During 2014, of 156 included febrile cases, 85 (54.48%) were Dengue monoinfection, 55 (35.25%) isolated Malaria, and 16 (10.25%) coinfection cases, whereas in 2015, of 417 febrile cases, 272 (65.2%) were Dengue, 117 (28.05%) isolated Malaria, and 28 (6.7%) coinfection cases. The coinfection and Dengue groups presented with a similar clinical picture. Among compared laboratory parameters, transaminitis was statistically significant in the coinfection group (p<0.001). Anaemia was significant in the Malaria group, whereas the Dengue group presented with raised haematocrit and thrombocytopenia. The coinfection group, with low haemoglobin and haematocrit, was consistent with concurrent Malaria coinfection. Among compared severity parameters, bleeding manifestations, renal dysfunction, and jaundice, was notable in the coinfection group, compared to the Malaria group (12% & 3.6% and 6.3% & 3.6% each respectively) with 3 mortalities in the Malaria and 1 in the coinfection group during 2014. During 2015, despite increased Dengue and coinfection cases, numerically, with increased jaundice and bleeding manifestations (16% &8% and 8% & 6% respectively), recovery was total.

**Conclusion:** Dual infection by Dengue and Malaria was observed by us for the first time in 2014. This phenomenon was noticed to recur subsequently during 2015 as well, and therefore merits further studies. Awareness and thus routine testing for both helps in effective management and reducing mortality as observed.
Melioidosis: An underdiagnosed entity in Odisha. A series of four cases over a two months period

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Background: With increasing awareness of melioidosis, increasing number of cases are now being reported from various regions of India, particularly South India. There is only one published report from Odisha, a state in Eastern India, till date. We report four cases of melioidosis, two septicemic and two localized cases, which presented to our hospital, over a period of two months.

Methods & Materials: Case 1: A 42 years old man, was referred our hospital with high grade fever and worsening abdominal pain in the left hypochondrium for last one month. CECT of the abdomen revealed splenomegaly with innumerable abscess cavities. B. pseudomallei was isolated from blood and splenic aspirate and was sensitive to Ceftazidime, Imipenem, Meropenem, Co-trimoxazole and Amoxicillin-clavulanic acid. Despite treatment with I.V ceftazidime and other supportive therapy, patient developed rupture of abscess to peritoneal cavity and emergency splenectomy was done. The patient expired on the 2nd post-operative day due to sepsis and cardiac arrest.

Results: Case 2: A 58-year-old diabetic patient presented with fever and cough of 3 months and worsening breathlessness and abscess in the back for a fortnight. Chest x ray revealed empyema thoracis. B pseudomallei was isolated from blood and pus. He was started on intravenous (IV) ceftazidime for 2 weeks and currently on suppressive therapy with trimethoprim–sulfamethoxazole. At this time he remains asymptomatic.

Case 3&4: Two cases mimicked tuberculous cervical lymphadenitis with raised ESR. Pus from lymph node aspirate yielded pure growth of B. pseudomallei in both cases, following which appropriate treatment for melioidosis was instituted. Both patients made a dramatic recovery.

Conclusion: We want to emphasize here on three important aspects, early clinical suspicion, prompt laboratory diagnosis and adequate intensive phase therapy. Chronic melioidosis often mimics tuberculosis and needs microbiological evaluation for proper management. Failure to respond to ceftazidime in the first case despite in vitro susceptibility is a cause of huge concern. A combination of a high index of suspicion, culture confirmation, early recognition of sepsis, and aggressive management of intensive phase with parenteral ceftazidime is necessary. A prompt switch to carbapenem is indicated if the clinical condition worsens with the administration of ceftazidime.
Genetic diversity of Orientia tsutsugamushi strains from patients in North India

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Background: Scrub typhus has emerged as a major cause of acute febrile illness in India in recent years. The causative agent, O. tsutsugamushi has more than 20 antigenic types due to a variable 56-kDa outer membrane protein. It is crucial to know the prevailing antigenic types in India for the success of diagnostic immunoassays and prospective vaccine candidates. In north India, the principal antigenic types circulating are unknown. Our tertiary care hospital caters to a very large area of north India (around 8 states with ten million population). Therefore, the current study was planned to identify the genotypes of O. tsutsugamushi isolated from patients presenting to our hospital from this wide area of north India.

Methods & Materials: All patients with AFI presenting to the hospital between July 2013 and December 2013 were included in this study. Whole blood was collected from patients for PCR. DNA was extracted using a DNA extraction kit. A nested PCR was used to amplify a 1003-bp and 483-bp region of the 56-kDa antigen gene using primers were taken from gene encoding for the 56-kDa antigen of the Gilliam strain of O. tsutsugamushi. The PCR products were purified and DNA sequencing was performed and aligned using the CLUSTAL_V program. A phylogenetic tree was constructed using neighbour-joining algorithms and analyzed using the sequences obtained in this study and those obtained from the GenBank database.

Results: Boryong-like strains pre-dominated overall and in all states studied (63.4%) followed by Karp-like (23.6%) and Gilliam-like (11.8%). We did not find any Kato-like strains and only one Kawasaki-like strain. Karp like strains showed >99% similarity to TH2033, TH2191, TH2208, Xinjiang & Neimeng strains and Gilliam-like strains showed >99% similarity to Clone ISS -11, Hualien 1, S072.

Conclusion: A previous study using PCR from eschars from south India showed a predominance of Kato-like strains but we could not find even a single Kato-like strain. Boryong-like strains predominated in our study. This shows that there is a huge diversity of Orientia tsutsugamushi in India. Boryong strains should be included in diagnostic assays as well as vaccines for scrub typhus, especially for north Indian populations.
Emerging rickettsioses in Northeast India
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Background: In India, rickettsial infections were first reported from Assam and West Bengal lay back during 1947. Since then, sporadic cases have been reported from different parts of the country except in the north eastern (NE) states. However, after a gap of 68 years, scrub typhus was reported to resurge in three states of NE region in 2012. This long serenity may be due to lack of surveillance and low index of suspicion for scrub typhus and other rickettsial infections. Therefore, a two year study was undertaken to study the sero-epidemiology of rickettsial infections viz., Scrub typhus (ST), Spotted fever group rickettsia (SFGR) and typhus group rickettsia (TGR) in Northeast India.

Methods & Materials: We investigated scrub typhus reporting areas of three states viz., Assam, Arunachal Pradesh and Nagaland during 2013-2014. About 908 human blood samples were collected from healthy volunteers with informed consent. All collected human sera were screened for antibodies against ST, SFGR and TGR using a four step indirect ELISA with respective antigens. A sample whose total net absorbance was ≥ 1.000 was considered as sero-positive. Positive samples were further titrated using 1:100, 1:400, 1:1600 and 1:6400 sample dilutions.

Results: Overall, 33.5% (305/908), 11.2% (102/908) and 3.9% (35/908) were found to be seropositive for ST, SFGR and TGR respectively. Co-circulation of ST and SFGR was found in all the three states whereas TGR were detected in samples collected from Arunachal Pradesh only. People engaged in agricultural and forest sectors were predominantly affected with a higher male to female ratio. Rural setting and lack of hygiene was a notable feature in the affected areas.

Conclusion: Our findings indicated a wide circulation of rickettsial infections in this region. This is also the first evidence of SFGR and TGR circulation in NE region of India. Continuous surveillance, understanding eco-epidemiology of these diseases and consideration of these agents for diagnosis of febrile illness by public health workers is warranted in future.
Predictors of severity in dengue infection
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Background: Dengue epidemic has become a serious emerging infectious diseases and a major public health problem in India. Some of the Dengue Fever patients present with mild symptoms and some present with severe manifestations involvement of vital organs responsible for high morbidity and mortality. Exact pathogenetic mechanism of severity is still not clearly understood. It is hypothesized that high level of serum TNF-alpha, IL-6 and soluble Thrombomodulin are associated with severe Dengue Fever, bleeding and shock.

Methods & Materials: It was a prospective observational case control study conducted at tertiary care hospital. All cases with either +ve for IgM or NS1 were enrolled for the study. Patients with shock were enrolled as cases and patients without shock were enrolled as control. We estimated serum TNF-alpha, IL-6 and sTM among shock and non-shock patients at the time of 1st visit and followed up till death, discharge or 14 days.

Results: Thirty Dengue patients with shock were enrolled as cases and 50 patients without shock were enrolled as control. Mean value of sTM was 15.8±6.4 ng/ml among shock and 6.4±4.4 ng/ml among non-shock and their difference was highly statistically significant (p<0.001). About 21 cases (70%) from shock and 3 cases (6%) from noshock were found to have high sTM level (>15 ng/ml) and their association was also found to have statistically significant (<0.001). About 12 cases (39.9%) from shock and 10 cases (20%) from non-shock were found to have elevated TNF-alpha (>100 pg/ml). About 11 cases (37.7%) from shock and 15 cases (30%) from non-shock were found to have elevated IL-6 (>100 pg/ml). However serum level of TNF-alpha and IL-6 were not found to be significant. We found 22% incidence (11) of shock among Dengue Fever and found to have significant rise of sTM (13±4.67) and IL-6 (136±76.4) at the time of 1st visit and they all had normal blood pressure but subsequently developed shock.

Conclusion: In our study we found high serum soluble Thrombomoduline level (sTM) and IL-6 were both predictor and risk factor for the development of severe Dengue Fever and shock.
A qualitative risk assessment of emerging infectious diseases of Bangladesh
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\textbf{Background:} Being an emerging disease hotspot, Bangladesh has lack of relevant data that allowed to assess risks of the introduction of emerging disease in the country. Thus, to fill up this knowledge gap, a qualitative risk assessment approach has been adopted to transform the tacit knowledge of subject expert into an explicit knowledge to update the current knowledge on disease management.

\textbf{Methods & Materials:} Two assessors from each discipline- animal health, human health and wildlife health- was invited to participate in an iterated qualitative approach to order the risk of emerging diseases in Bangladesh. Definition and scores of “likelihood” and “consequence” were adopted from the Australian standard for risk analysis. The results of each assessor were assembled into SRA version 3.0 tool. Spearman’s rank correlation coefficient (ranges between -1 and +1) was used to identify the agreement and disagreement level between assessors. This tool has the hazard rank graph to distinguish hazards that prevail the majority of uncertainty.

\textbf{Results:} Assessors correlations was moved from 0.14-0.87 to 0.49-0.85 in second risk assessment. However, improved correlation between assessors was seen in second assessment. Median correlation was rose from 0.51 to 0.65 in second assessment. Importantly, no negative values was recorded between pairs of assessors signifying no high disagreement in hazard rank order among assessors of three field (Figure 1 and 2). “HPAI” and “Nipah virus” were considered the two most important hazards of the 11 hazards, while “Lyme disease” was found the least important hazard in the country. Subsequently, high and narrow uncertainty level score were evident with “Nipah virus” and “Lyme disease” portraying significant agreement and disagreement among assessors (Figure 3).

\textbf{Conclusion:} Though no significant agreement and variability in the perceived risk was emerged, an iterated qualitative approach can provide an interim quality data, by reducing the linguistic uncertainty among assessors, to guide policy makers to formulate necessary steps for disease mitigation. Further testing and validation through various examples and scenarios are still required to adopt this method in developing countries like Bangladesh.
Group B streptococcus: An emerging infection in South Asia
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Background: The disease burden of neonatal group B streptococcal (GBS) disease is substantial but gaps remain in defining the global impact of this infection. Determining the prevalence of GBS colonization during pregnancy in South Asian countries can provide important information because 1% to 2% of infants born to women colonized with group B Streptococcus at delivery develop early-onset GBS disease unless intrapartum antibiotic prophylaxis is provided to interrupt transmission. When optimal methods are employed, including obtaining cultures from the lower vagina and rectum and processing samples in selective broth media, maternal GBS colonization rates range from 20% to 35%. There is limited information regarding GBS maternal colonization rates from studies conducted in South Asia.

Methods & Materials: To review studies conducted in South Asia (India, Pakistan, Bangladesh, Sri Lanka, Nepal, Bhutan, Afghanistan, Maldives) PubMed search with search words: Group B Streptococcus AND maternal colonisation AND prevalence AND name of each country in South Asia

Results: Table: Maternal Group B Streptococcal Colonization Rates: South Asia

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Timing</th>
<th>Number of women</th>
<th>Sites†</th>
<th>% positive</th>
<th>Selective enrichment broth**</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Chaudhary/1981</td>
<td>Labor</td>
<td>100</td>
<td>T, V</td>
<td>16 (V:10, T:6)</td>
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<tr>
<td>Mani/1984</td>
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<td>325</td>
<td>V, E</td>
<td>5.8</td>
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<tr>
<td>Lakshmi /1988</td>
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<td>207</td>
<td>HV</td>
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<td>NS***</td>
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<tr>
<td>Kulkarni/1999</td>
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<td>Dalal/ 1998</td>
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<td>Bangladesh</td>
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<td>Chan/ 2011</td>
<td>Pregnancy/Labor</td>
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<td>V, R</td>
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<td>HV, R,</td>
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<td>V</td>
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<td>Najmi/2013</td>
<td>Pregnancy</td>
<td>405</td>
<td>HV</td>
<td>17</td>
<td>NS</td>
</tr>
</tbody>
</table>

†T, throat; V, vaginal; HV, high vaginal; R, rectum; E, endocervix ; P, perineal ; U, urine

**Todd-Hewitt broth with gentamicin and naladixic acid

***NS, not specified

Conclusion: Existing reports cite a broad range in GBS colonization prevalence during pregnancy in South Asia. Studies to date have varied in choice of sites sampled, use of selective broth media and sample size. Available evidence likely underestimates the prevalence of maternal GBS colonization. Unmet need for
prospective studies that employ optimal methods to determine accurately the prevalence of GBS colonization in pregnant women in South Asia
Comparative risk factors for nosocomial non-carbapenemase producing (NCP) and carbapenemase producing (CP) carbapenem resistant Enterobacteriaceae (CRE) intestinal colonization

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Background: Carbapenem resistance is an emerging global problem. Resistance to carbapenems arises through either acquisition of resistance genes (IMP, NDM-1, KPC, OXA) or production of extended spectrum beta-lactamase with alteration of porin expression. In Singapore, there has been an increase in CRE since 2010. The primary objective of this retrospective case-case-control study is to identify and compare risk factors for colonization with NCP CRE and CP CRE.

Methods & Materials: Patients admitted between September 2010 and July 2013 to Tan Tock Seng Hospital, a teaching hospital in Singapore, were included. We selected three groups of subjects. Case 1: patients who had NCP CRE gut colonization. Case 2: patients who had CP CRE gut colonization. Control: Patients who were screened negative for CRE. Demographic, clinical, microbiological data and antibiotic usage in preceding 90 days were collected from electronic medical records. Statistical analysis was done using STATA 12.0.

Results: 1934 patients were screened for CRE, of which a total of 17 patients colonized with NCP CRE and 15 patients with CP CRE (13 NDM-1 and 2 OXA-48) were compared with 60 randomly selected control patients. Of the NCP CRE, 9 were Klebsiella pneumoniae, 1 was Enterobacter cloacae, 1 was Enterobacter aerogenes and 6 were not speciated. Of the CP CRE, 7 were Klebsiella pneumoniae, 4 were Escherichia coli and 4 were Enterobacter cloacae. There was no significant difference in age, sex and Charlson index between cases and controls. Duration of fluoroquinolone and carbapenem exposure was significantly longer for NCP CRE compared to controls: fluoroquinolone-(3.88 ± 6.47 vs 1.00±2.80; OR, 1.16; 95% CI, 1.02 – 1.32; P=0.02) and carbapenems- (5.29 ± 7.70 vs 1.62 ± 5.52; OR, 1.08; 95% CI, 1.00 – 1.17; P=0.05). Of note, antibiotic exposure in the preceding 90 days was not associated with CP CRE colonization.

Conclusion: While preceding antibiotic use was a risk factor for NCP CRE colonization, it was not found to be a risk factor in CP CRE colonization. As such, we will need to continue to evaluate risk factors and infection control strategies for CP CRE.
Molecular identification of nontuberculcus mycobacteria in humans in Zimbabwe

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Background: Several nontuberculcus mycobacteria (NTM) have been isolated from diverse environments such as water, soil, sewage, food and animals. Some of the mycobacteria are now known to be opportunistic pathogens in humans. In this study, we describe the molecular identification of NTM in humans in Zimbabwe.

Methods & Materials: Human sputum samples were collected during the national TB survey that was carried out by the Ministry of Health and Child care of Zimbabwe in 2014. The NTM were isolated using Lowensen Jensen (LJ) media and tested for growth in the presence of ParaNitroBenzoic acid and their ability to grow at different temperatures, 25°C, 37°C and 45°C. DNA was extracted and rRNA gene amplified by PCR. Amplicons were sequenced and analyzed using bioinformatics. Species were identified.

Results: Out of total of 963 NTM isolates from sputum samples, 81 were analyzed using 16S ribosequencing. Forty isolates (49.4%) were found to belong to Mycobacterium avium complex (MAC) species. The other 41 isolates (50.6%) were identified as M. lentiflavum (6.2%), M. terrae complex (4.9%), M. kansasii (3.7%), M. moriokaense (3.7%), M. asiaticum (2.5%), M. novocastrense (2.5%), M. brasiliensis (2.5%), M. elephantis (2.5%), M. paraffinicum (1.2%), M. bohemicum (1.2%), M. manitobense (1.2%), M. intermedium (1.2%), M. tuberculosis complex (1.2%), M. parakoreense (1.2%), M. florentinum (1.2%), M. liitorale (1.2%), M. fluoranthanivorans (1.2%), M. sherrisii (1.2%), M. fortuitum (1.2%) and M septicum (1.2%). Two isolates (2.5%) could not be identified, but were closely related to M. montefiorens and M. phlei respectively.

Conclusion: Interestingly, the MAC species were the commonest NTM during the survey. Further studies are necessary to ascertain the true diversity of NTM in Zimbabwe.
Ethio-nosologic opportunism in tropical, emergency and re-emergency diseases in Albania

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Background: It is now scientifically proven ability of tropical, emergency and riemergency diseases to kill human immunity and create a bed for generating optimal opportunistic diseases in these subjects. Need to precede problematic in question, it brought the opening of the first and the only cabinet of tropical diseases in Albania. So far little is written in this field.

Aim of study is to note their option and bringing our experience in this direction.

Methods & Materials: Material: Included 321 subjects from which underlined 239 subjects with depressed immunity, age group 14-71 years old, during the period 1995-2015. The diagnosis of opportunistic pathologies is determined based on relevant protocols clinical, biological imaging, and standard microbiological, CD4 + immune research, leukocyte immunophenotype, tumor markers.

Methods: The study is the analytical type, retrospective and prospective. For every patient we cheked possible opportunistic infections during their. Tropical diseases we included malaria 25 case, multiple cutaneous leishmaniasis imported from Iran 1; Emerging infectious diseases: 30 cases with AH1N1 Flu, HFRS 15, WNV 6; Pneumocistosis on HIV/AIDS 51, TB in AIDS (Pulmonary 23, lymphadenitis 2, meningitis 1). Re-emerging infectious diseases. Syphilis 36 on HIV/AIDS, Leishmaniasis in AIDS 6, Brucellosis 12 (column 7, valvular prosthesis 5); discitis 14 (6 on TB, 3 staff aureus, staff epidermitis, 1 enterococcus spp). Sepsis 5 (2 of staff aureus, 2 from staff epidermitis, 1 staff resistant from vancomycin). Visceral Leishmanisis 10, (6 HIV/AIDS, 4 on TB). 2 case of CMV on HIV/AIDS.

Results: Opportunistic infections displayed on tropical, emergency and re-emergent diseases comprised of: Encefalitis 9 cases (malaria 3, WNV 6, Influenza AH1N1 1), pneumonia 99 (25 AH1N1, 51 pneumocistosis, 23 TB on HIV/AIDS), discitis 14 (35 TB, 4 Staf.aureus, 3 staff epidermitis, 1 enterococcus spp), Endocarditis 3 (brucellosis), Hematological infections 6 (sepsis by staff aureus 2 cases, staff, epidermidis 2, vancomcin resistant 1, visceral leishmaniasis 1), lymphadenitis 2 (HIV / AIDS). Gastro-intestinal infection 37 (35 candidiasis, 2 CMV, 1 TB on HIV / AIDS).

Conclusion: 1. Ethio-nosologic structure of opportunistic pathologies included 12 causes.
2. Topography of opportunistic pathologies related of 7 organs affected: CNS 9, Pulmonary 99, vertebral column 14, haematopoietic system 6, cardiac 5, lymphatic system 2, gastrointestinal 37 cases.
Sri Lanka: Nationwide epidemiology of melioidosis  

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**Background:** Sri Lanka lies in the melioidosis belt. Most of the country is rural. Rice farming, using traditional methods, is prevalent. Rice fields are the natural habitat of *B. pseudomallei*. Melioidosis has been compared with an iceberg, since the majority of cases are hidden. The status of melioidosis in Sri Lanka is uncertain.

**Methods & Materials:** National surveillance was instituted, with a network of microbiology laboratories and a standard case definition, laboratory work up procedure and questionnaire. Case detection was improved by raising physician, microbiologist and technician awareness. International collaboration with PathWest in Western Australia was established.

Primary isolation relied on conventional culture. Suspected isolates were referred to the reference laboratory for bacteriological identification and confirmation by real time PCR assays for *LpxO*.

**Results:** Only three cases had been reported between 1927 and 2005. From 2006-15 we detected 90 cases. The number rose every year, with 70% detected in 2014/15. Mortality declined, with an overall mortality of 20%. Cases presented throughout the year with two peaks during monsoons. Melioidosis was prevalent throughout the island, but an infection-free area comprising the highlands above 500m, where the main crop is not rice but tea, emerged.

A majority of cases were middle-aged men, corresponding to likelihood of soil exposure and onset of diabetes. While men (74%), rice farmers/cultivators (51%) and rural populations (82%) predominated, housewives, schoolchildren, professionals, businesspersons and white collar workers were represented. The wide age range (2-92 years) reflects ubiquity of exposure. Melioidosis in Sri Lanka appears to be related to the outdoor, agricultural, barefoot lifestyle practiced by the majority of the population. Lower limb involvement was common, which may point to the site of bacterial inoculation consistent with a barefoot lifestyle. While diabetes, organ disease and thalassemia were significant risks, melioidosis was seen in healthy adults and children probably due to high dose exposures.

**Conclusion:** Active surveillance has established that melioidosis is endemic in Sri Lanka, with a wide geographic and demographic distribution. Improved diagnosis has led to reduced mortality, most probably through earlier diagnosis and better targeting of treatment. There is an urgent need to extend these benefits to relatively under-resourced parts of the country.
Identification of bartonella spp and rickettsia spp of human body lice from homeless people of Bogota D.C, Colombia
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Background: Human body lice (Pediculus humanus humanus) is recognized as the vector of different pathogenic bacteria as Bartonella quintana and Rickettsia prowazekii, the etiological agents of trench fever and epidemic typhus, respectively, being these bacteria re-emerging pathogens in different countries. Poor living conditions and limited access to public services are predisposing factors to increase high prevalence of body lice infestation in different populations such as homeless people. In Bogota-Colombia, approximately 9,614 homeless people are living in unsanitary conditions. However, our country has no reports about the presence of louse-borne bacteria and infection caused by this. Therefore, the aim of this study was to establish the presence of Bartonella spp and Rickettsia spp in body lice collected from homeless people in Bogota.

Methods & Materials: A total of 201 body lice were collected from 18 individuals. Lice were taxonomically classified and pooled by host and development stage. 39 pools were obtained for the DNA extraction and all samples were subjected to PCR. For the identification of Bartonella spp, PCR was made to amplify a part of the citrate synthase (gltA) and ITS1 genes and for Rickettsia spp we amplified the gltA, ompB and 16SrRNA genes.

Results: A total of 11 (28.2%) Bartonella spp lice pools were positives, from these 7 were positive for gltA, 10 positive for ITS1 and 6 positive for both genes. Also, 34 lice pools (87.1%) were positives for Rickettsia spp (gltA, ompB and 16SrRNA genes were identified 42 [62%], 1 [3%] and 17 [44%] pools positives, respectively), 6 pools positives for two genes (gltA and 16SrRNA) and 1 positive for three genes. Furthermore, 9 lice pools (23%) were positives for both microorganisms. 9 (50%) and 17 (94%) homeless individuals were positives for Bartonella spp and Rickettsia spp, respectively. While 7 (38.8%) were positives for both microorganism.

Conclusion: This is the first report in Colombia and South America detecting Bartonella spp and Rickettsia spp in human body lice from homeless people, indicating that they are present in this population and create a risk for public health in Colombia, especially in susceptible populations with poor living conditions.
Persistence of Japanese encephalitis virus infection in healthy children in JE Endemic Area

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Background: Japanese encephalitis (JE), a vector-borne disease that causes neurological infections in humans especially targets children below 15 years. Despite of introduction of JE vaccine there has been increase in the number of cases of AES cases with high case fatality rates. The present study was conducted to assess the risk of JE through research on animal-vector-human interactions and assess the cellular immune response in JE vaccine responders and non responders.

Methods & Materials: A cross sectional was conducted in 12 villages (4 each in high, medium and low burden blocks) of the high endemic Kushinagar district, Uttar Pradesh, India in 2012. Detection of virus was done by real time RT-PCR in children and IgG testing in younger cohorts of pig. To assess the immune response 5 ml blood was collected from 149 children pre and post vaccination. Peripheral blood mononuclear cells were prepared by using cell preparation tube. Antibody titration was done by plaque reduction neutralization test (PRNT). CD3, CD8, CD4 Th1, CD4Th2 and Treg immune cells were measured by flow cytometry.

Results: 10/151(6.6%) CSF samples collected from patients were positive for JE infection and 5 were positive for Enterovirus by realtime RT-PCR. In 22/275(8%) of healthy control children the pre-existing JE neutralizing antibody representing subclinical infection were observed by PRNT. 8/50(16%) of pigs were positive for JE by IgG ELISA. 23/149(15.43%) healthy children had antibody titer below 10 and were vaccine non-responders. Treg expansion was found in the non responders group and difference was statistically significant. Most vaccinee belonged to moderate titre group with antibody titer 80 to 160.

Conclusion: The study emphasizes the need for high quality of surveillance both in terms of isolation virus and non cultivable virus by third generation of sequencing. SA-14-14-2 is capable of inducing humoral and cellular immune response. The study suggests the role of expansion of Treg activity which inhibits humoral immune response, thus may contribute to persistent infection. There is need for extensive research in areas related to surveillance, vaccines and virus persistence to provide better understanding of vector borne diseases.
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Background: Diphtheria has not been recorded in Gia Lai province since 2004. Beginning in October 2013 a large number of suspected cases were reported from K’Bang District hospital, including two deaths. This study describes the epidemiological characteristics of these cases.

Methods & Materials: Retrospective review of patient medical records, and face-to-face interviews of health care providers and patient care-givers.

Results: 108 suspected cases of diphtheria, including two deaths, were reported from K’Bang district from October 2013 through July 2014. Specimens from 7 of 16 cases tested were culture positive for Corynebacterium diphtheria. Among the 108 cases, 73% were 1-15 years-old, none were <1 year-old; 84% belonged to the Bana ethnic minority group; 93% were from farming families and 71% were poor; 69% of care givers were illiterate. District wide, only 39% of all families are Bana, 85% are farming families, 30% are poor, and literacy is 95%. Most cases (87%) had not received or had an unknown history of DPT vaccination. Cases came from 13 of the 14 communes in the district, but the largest number (39%) was from Daksmar where the index case lived. Recent coverage with DPT-3 in Daksmar has been the lowest reported for any commune in the district, <50% (2006-2007) and <76% (2010-2014).

Conclusion: The diphtheria outbreak in K’Bang from 2013 through 2014 disproportionately affected children <15 years old, an ethnic minority group, and children of the poor, and illiterate. Rates of immunization in this community have been very low in recent years. Future strategies to improve vaccination coverage in the area should include targeting of poor minority communities and include materials for the illiterate.
Ebola virus disease signs, symptom and predicting death: A literature review

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**Background:** The aim of this study is to determine the accuracy of signs and symptoms among patients with Ebola at the time of presentation in predicting deaths.

**Methods & Materials:** We did a literature review in PubMed using the search terms or combinations of the search terms “ebola”, “death” “signs and symptoms”. Articles published between January 1976 and March 2015, have been include in this analysis. Further papers were found by screening the reference lists of these articles or as relevant articles suggested by Pubmed. Data are presented using likelihood ratio (LRs) because they function as “diagnostic weights” that are easily translated to posttest probability of death.

**Results:** Three studies were identified (Democratic Republic of Congo (DRC) =2 [1 from Kikwit and 1 from Mosango during the 1995 Outbreak Ebola Hemorrhagic Fever (EHF)], Sierra Leone=1 during the 2014 outbreak Ebola Virus Disease (EVD). A total of 103 cases have been analyzed in the study from Kikwit with 84 patients who died and 19 patients who survived, 23 cases were identified in Mosango; 18 of them died. In Sierra Leone, 44 cases among them 36 died. Several findings significantly decrease the probability of dying of Ebola: In Kikwit, DRC; the presence of Cough (likelihood ratio [LR]:0.27; 95% confidence interval [CI], 0.09 - 0.80), hemoptysis (LR:0.08; 95% CI: 0.01-0.74) and the absence of Tachypnea (LR:0.72; 95% CI: 0.61-0.86). In Mosango, DRC; the absence of: Conjunctival injection (LR:0.19; 95% CI: 0.04-0.82), Melena (LR:0.53; 95% CI: 0.30-0.93) and the presence of Rash (LR:0.12; 95% CI:0.03-0.45) significantly decrease the probability of dying of Ebola. In Sierra Leone, the absence of: asthenia (LR:0.44; 95% CI:0.27-0.72), diarrhea (LR:0.60; 95% CI: 0.40-0.90), dizziness (LR:0.51; 95%CI:0.32-0.80) and the presence of Hearing loss (LR:0.06; 95%CI:0.01-0.23) significantly decrease the probability of dying of Ebola.

**Conclusion:** The probability of the signs and symptoms of Ebola Virus Disease in predicting death varies from one center to another during the same epidemic and an epidemic to another. Further studies need to be conducted in this direction.
Drivers for MERS-CoV emergence in Qatar


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Background: The recent discovery of MERS CoV as a zoonotic disease endemic in dromedary camels raised questions about the possible origin of this infection. In addition, it remains unclear why and how MERS-CoV transmission occurs exactly and many questions remain concerning the ecology of this virus in relation to human exposure. Therefore, we set out to review the history and statistics of camel farming in Qatar, in order to generate hypotheses about the drivers for MERS CoV emergence and human infection.

Methods & Materials: Based on initial interviews and brainstorming within the joint Qatar-NL MERS CoV outbreak investigation team, a list of possible determinants and contributing factors of MERS CoV emergence was drafted. Available statistics and literature was reviewed. The available data was used to generate hypotheses about the factors leading to emergence of MERS CoV as a human health threat.

Results: Over the past 40 years, the practices of animal ownership, herding and farming have changed considerably. With the discovery of oil in 1940, major changes occurred in lifestyle and wealth. The increased wealth led to huge increase in ownership of camels, mainly for racing activities. The total camel population increased from 4300 in 1992 to over 84000 in 2015. As a precious animal, camels are being kissed, hugged, and greeted. Consistent with the disease seasonality, contact with animals intensifies during winter time where camel-related activities flourish through race and beauty competitions, trade, and breeding. These activities imply extensive camel movement and mixing along with their owners and workers from all over the Gulf region. Moreover, camel products are used for a variety of domestic purposes. Camel-workers live inside camel barns while owners pay regular visits to their barns. Nevertheless, they all strongly deny that MERS-CoV can be transmitted from camels to humans.

Conclusion: The rapid increase in GDP in the past two decades has been paralleled with rapid growth of the camel trade and racing industry. Current practices of people around camels involve frequent contact with camels which might provide an opportunity for the infection transmission. More in-depth studies were needed to understand the role of social practices in the virus transmission.
Corynebacterium diphtheriae: An emerging cause of chronic suppurative otitis media

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**Background:** *Corynebacterium diphtheriae* is an established upper respiratory pathogen associated with infections that range from mild localized lesions to severe life threatening toxemia. Pharyngeal mucosa is the most frequent site affected than any other sites. The incidence of disease has drastically reduced in recent years due to improved immunization coverage in children. However, it resulted in characteristic shift in the age distribution to adults as well as superseding of toxigenic strains by non-toxigenic strains of *C. diphtheriae*. As a consequence, non-toxigenic strains are increasing being found to be associated with carrier state and infections of other body sites like skin, in addition to pharyngeal infections. Involvement of middle ear leading to development of Chronic Suppurative Otitis Media (CSOM), however, has been rarely implicated in the available literature.

**Methods & Materials:** Herewith we report three cases of CSOM within a span of six months caused by *Corynebacterium diphtheriae*. Patients were young adults, without any underlying immunodeficiency with complete immunization in childhood. They presented with complaints of discharge from one/both ears and reduced hearing since a long period (range 3 - 8 years). Aural examination revealed a perforation in tympanic membrane without obvious pseudomembrane formation and with purulent discharge. There were no signs of toxemia or systemic involvement. Discharge material was sent for microbiological testing.

**Results:** Gram and Albert’s staining of discharge showed presence of bacilli morphologically suggestive of *C. diphtheriae*. Culture on potassium tellurite medium grew black colonies after 48 hours of incubation, later identified as *Corynebacterium diphtheriae biotype mitis* by standard microbiological techniques. The toxigenicity testing performed at National Centre for Disease Control, Delhi revealed one isolate as toxigenic and other two as non-toxigenic.

**Conclusion:** Increased and concurrent isolation of toxigenic as well as non-toxigenic strains of *C. diphtheriae* from relatively uncommon sites like ear, within a short time span is a potentially alarming finding which demands more clinical awareness in healthcare providers. It may implicate possible underestimation of actual cases since large population is treated empirically at private sector without being properly investigated. Also, possible unpredictable conversion of non-toxigenic strains into toxigenic may pose a significant threat for unimmunized individuals in the community.
Lessons learnt from a recent Ebola virus outbreak: A scoping study

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Background: Ebola outbreak of West Africa (2014) is the longest reported outbreak which recorded 6553 cases and 3083 deaths till 30th September 2014. It brought out the prevalent problems of poor preparedness and inadequate public health response in the affected countries. It is important to understand what factors were responsible for this unprecedented outbreak and not repeat the same mistakes in future disease control measures in West Africa and elsewhere.

Methods & Materials: Scoping study to summarize range of evidence available on current Ebola outbreak was done. All articles in English language related to epidemiology of Ebola in humans, published between 1st March and 30th September 2014 were considered for review. Search engines like PubMed and Google Scholar were used to search key word “Ebola”, “Ebola Virus”, “Ebola Viral Disease” and “Ebola Hemorrhagic Fever”.  
Snowballing with cross references was done to find related literature on Ebola. Related websites, blogs and published news articles were reviewed. After review of 142 articles from Pubmed and Google Scholar, 58 comprehensive articles were selected and rest were excluded on the basis of theoretical saturation of the published material.

Results: This is the first ever Ebola outbreak affecting large urban communities. Factors which worsened the outbreak were: weak health systems, un-favorable cultural practices, poverty, illiteracy, mistrust for government, extensive cross border movement, slow and inadequate response from international agencies and lack of tested prevention strategies. High case fatality fueled a misunderstanding that being taken away by medical teams to treatment centers means certain death. As a result, simple measures of universal precaution, isolation and tracking of contacts, supportive treatment and appropriate burial practices were difficult to implement.

Conclusion: This outbreak illustrates serious weaknesses in the international community’s ability to respond to outbreaks. Cost of setting up an infrastructure for early effective response is insignificant compared to huge social and economic cost of the outbreak. Also essential are a strong health system and effective community participation. Even when the understanding of disease epidemiology is not crystal clear, simple supportive interventions delivered timely and efficiently can compensate for the lack of effective vaccines or antimicrobials.
Occurrence of dengue in 2013 and 2014 in northern Mozambique: Is dengue an endemic disease in Mozambique?

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Background: Dengue is the most rapidly spreading vector borne viral infections worldwide and represents a leading cause of fever in several countries. It’s estimated that two-fifths of the world population is at risk of infection. Recent outbreaks of dengue were reported in two consecutive years (2013 and 2014) in two provinces situated in northern Mozambique. In this abstract we present main findings of both outbreaks and correlated this with possible endemicty.

Methods & Materials: Provincial health authorities from two provinces situated in northern Mozambique, namely Cabo Delgado and Nampula reported the occurrence of cases suspected of Dengue. After investigation, Dengue outbreak was confirmed. During these outbreaks, samples were initially tested by RDT at the health unit, and positive samples were shipped for further testing at the reference laboratory in Maputo. Entomological investigations were also conducted.

Results: A total of 193 suspected cases were reported in 2013, of which 100 were Dengue positive. In 2014, a total of 605 suspected cases were reported, of which 171 were Dengue positive. Entomological investigations conducted in both years also found high density of Aedes mosquito in the capital city of both provinces. All suspected cases were reported in the urban and suburban area of the capital cities of both provinces. Dengue cases occurred during raining season with peak in March. No serious case of Dengue was detected. Serotype specific PCR confirmed infection by Dengue-2 serotype.

Conclusion: In conclusion, data from two recent Dengue outbreaks in consecutive years in northern Mozambique, suggest that the disease has become endemic in Mozambique and likely will continue to occur at increasing frequency if no control measures are put in place. More studies for better understanding of the epidemiology, geographical extension and burden of Dengue in Mozambique are urgently needed. These studies will provide evidence for the rapid definition of control measures in Mozambique.
Fluoroquinolone resistance in Shigella over a decade in India: Do we have Plasmid-mediated quinolone resistance?

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**Background:** The qnr gene family consists of plasmid mediated quinolone resistance (PMQR) genes that confer low level resistance to nalidixic acid and reduced susceptibility to ciprofloxacin. At our tertiary care referral centre in Chandigarh, which caters to a large population of 5 neighboring states, antibiotic resistance in Shigella is being constantly monitored.

**Methods & Materials:** We screened 139 S.flexneri and 38 S. dysenteriae serotype 1 isolated during 2001 to 2011, for PMQR (qnrA, qnrB, qnrS, aac(6′)-Ib-cr and qepA) genes. MICs were determined for nalidixic acid and ciprofloxacin by E-test. The isolates were classified as susceptible (MICs <0.5 mg/L) or resistant (MICs ≥1 mg/L) to ciprofloxacin; susceptible (MIC ≤ 16) or resistant (MIC ≥ 32) to nalidixic acid respectively. Those positive for PMQR were tested for ESBL (CLSI).

**Results:** Eleven (6.12%) isolates, were found to harbor PMQR, out of which 2 were positive for qnrS1 gene (S. flexneri), while 9 for aac(6′)-ib-cr gene (6 S. flexneri and 3 S. dysenteriae). No strain harbored both genes. None were positive for qnrA, qnrB, qnrD and qepA genes. Out of 11 PMQR positive isolates, 3 showed ciprofloxacin MIC of ≥32 mg/L, while 9 strains were resistant to nalidixic acid with a MIC of > 128 mg/L, 3 were positive for CTX-M-15 and 1 for CMY-2 gene. The aac(6′)-ib-cr gene was detected as early as 2002, combination of CTX-M-15 with aac(6′)-ib-cr appeared in 2005 and qnrS1 appeared recently in 2010. PMQR could not be the direct reason for quinolone and fluoroquinolone resistance in these strains. Sequencing of gyrA and parC revealed three mutations on the QRDR of each of the 11 PMQR positive strains, thought to be primarily responsible for quinolone resistance phenotype.

**Conclusion:** After description of qnrS1 Shigella in Japan, China and USA, we report the first description of qnrS1 Shigella in India. Furthermore, combination of PMQR and CTX-M-15 genes is being reported here for the first time in Shigella. The close association of aac(6′)-ib-cr, with CTX-M-15 is of great concern as CTX-M-15 has emerged worldwide. The two qnrS1 strains detected were from 2010, indicating a relatively new appearance among Shigella in India.
Background: There is an increase in H1N1 patients in our community leading to morbidity and mortality. This study aimed to assess the epidemiology and characteristics of H1N1 patients requiring intensive care unit admission.

Methods & Materials: The retrospective cohort study included 14 H1N1 patients admitted in ICU from November, 2014 to April, 2015. Data was analyzed from patient's medical records. All ICU patients who were tested positive for H1N1 by reverse – transcriptase polymerase – chain reaction assay were included. Patients treated for H1N1 in the ward and out – patient department were excluded. Categorical variables were compared using Fisher's exact test.

Results: In our study, the mean standard error (SE) age was 46.85 (3.85) years and male to female distribution was equal. Majority of the patients presented with dry cough (85.7%), dyspnea (78.6%) and fever (71.4%) on ICU admission. 92.8% patients had moderate to severe adult respiratory distress syndrome. Sequential organ severity assessment score was less than 11 for 92.8% patients. Non- invasive ventilation (NIV) was initiated on 78.6% patients and 6 (42.8%) patients improved on NIV alone. Eight (57.1%) patients needed invasive ventilation and all ventilated patients required prone ventilation within 24 hours. Mean duration of mechanical ventilation was 23.5 (7.21) days and mean duration of prone ventilation was 5.5 (2) days. Tracheostomy was done in 21.4% patients. Complication during ICU stay included ventilator associated pneumonia (75%), critical illness polyneuropathy (12.5%), acute kidney injury (14.3%) and pneumothorax (7.1%). Mean days of ICU stay was 14.7 (4.27) days and mean duration of hospitalization was 23.07 (7.61) days. The 30 days survival rate in our study was 64.3% and mortality was attributed to severe refractory hypoxemia.

Conclusion: H1N1 patients are unique in presentation and management. There is a need for awareness and education for early initiation of Oseltamivir at primary care centers to reduce the severity of H1N1. The patients required longer ventilation and all ventilated patients required prone ventilation. Extra corporeal membrane oxygenation could be considered as rescue therapy in severely hypoxic patients.
Campylobacter jejuni infection and Guillain-Barré syndrome: An emerging cause of acute flaccid paralysis after the eradication of poliomyelitis in Bangladesh

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Background: Bangladesh has achieved a remarkable success with the eradication of poliomyelitis. However, non-polio acute flaccid paralysis and its clinical presentation as the Guillain-Barré syndrome (GBS) is still frequently diagnosed. GBS has a diverse clinical phenotype that varies according to geography. Campylobacter jejuni enteritis is the predominant bacterial infection preceding GBS. The purpose of the study was to define the clinical phenotype of GBS and the relation with preceding C. jejuni infection and anti-ganglioside antibodies (GM1, GD1 and GQ1b) in a large GBS cohort from Bangladesh.

Methods & Materials: We conducted a hospital based observational study including 403 patients fulfilling the National Institute of Neurological Disorders and stroke (NINDS) criteria for GBS patients between 2010 and 2013 in Dhaka Medical College Hospital, Dhaka, Bangladesh. Detailed clinical, electrophysiological, serologic and microbiological data were obtained.

Results: GBS affected predominantly in young adults males (M/F=2:1) living in rural areas. Antecedent events were recorded in 76% of patients. The most frequent events being gastroenteritis (46%) and upper respiratory tract infection (18%). Sixty-five percent of the patients were bed-bound (Hugh’s F-score, 4) at entry and 17% patients required mechanical ventilator (F-score 5). Electrophysiological studies showed that 54% of patients had an axonal variant of GBS. About 90% patients did not receive specific treatment either Intravenous Immunoglobulin (IVIg) or plasmapheresis due to high expensive treatment cost. After 6 months follow up, 13% patients had died and 15% were still disabled. Evidence for a recent C. jejuni infection was found in 55% of GBS patients. In a GBS-associated strains collection from Bangladesh, capsular types HS23/36c, HS19, and HS41 were most prevalent. MLST analysis showed that HS19 (ST-22), HS23 (ST-3219) and HS41 (ST-362) were prevalent. Anti-ganglioside antibodies (GM1, GD1a and GQ1b) were frequently detected in GBS patients.

Conclusion: GBS in Bangladesh is a severe, predominantly pure motor and axonal neuropathy with a high mortality rate. Recent C. jejuni infection and anti-ganglioside antibodies accounted for a significant proportion of GBS. Majority of the patients do not receive specific and standard treatment with IVIg in view of its high price. Therefore, low-cost treatment strategies are required for GBS patients in developing countries.
Dengue sero-prevalence and serotype distribution among children near Hyderabad, India

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Background: India has reported cases of dengue for more than 200 years and in recent times this has burdened the healthcare system and challenged policy makers. It is estimated that dengue is substantially under reported, a fact corroborated by seroprevalence finding 50 – 89 % of adults have suffered at least one infection in their lifetime. Younger age is a risk factor for severe disease and in recent outbreaks, many cases have been observed in children. Co-circulation of multiple serotypes is also a risk for severe disease and the relationships between serotypes, attack rate and susceptibility are important to inform prevention and control activities, including vaccination.

Methods & Materials: A community-based, cross-sectional, seroprevalence study (CTRI/2011/12/002243) was conducted at 8 sites in India among apparently healthy 5-10 year old children from January 2011 to 2012. This analysis was conducted to improve understanding of dengue epidemiological parameters and environmental and demographic covariates from 2 sites located in southern India near Hyderabad. Dengue was tested by Indirect enzyme-linked immunosorbent assays (ELISA) and positive samples were subject to PRNT₅₀ to detect neutralizing antibodies against DENV-1, DENV-2, DENV-3 and/or DENV-4.

Results: The mean age of 640 subjects included in the analysis was 7.8 years. Dengue IgG seroprevalence was 58.3% (373 subjects with previous exposure and increased with age. Of PRNT profiles, 190 (50.9%) were multitypic, signifying >1 previous infection, 170 (45.6%) were monotypic, and 13 (3.5%) were seronegative. DENV2 & DENV3 predominated (29.5% and 40.0% of multitypic; and 41.8% and 27.6% of monotypic profiles), however, serological profiles against all four serotypes were identified. Serological outcome was not affected by sex, piped water supply to house, public sewer presence, water storage in house, previous dengue infection and family history of dengue infection.

Conclusion: The levels of previous exposure, the quantum and increasing trends with age and multiple serotypes circulating among children <11 years of age indicate transmission potency and risk of severe disease episodes following secondary infections, even in young children. There is an urgent need for appropriate interventions to control, diagnose and treat dengue, more sensitive public health surveillance and further research to identify the covariates in dengue disease.
Human ocular dirofilariasis due to *Dirofilaria repens*: an underdiagnosed entity or emerging filarial disease?

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Background: *Dirofilaria* are natural filarial parasites of dogs, cats and foxes. Human dirofilariasis is an accidental zoonotic infection caused by species *D. immitis*, *D. tenuis* and *D. repens*. Human ocular dirofilariasis were initially reported from Kerala. But for a solitary case of oral dirofilariasis, it has not been reported from Tamil Nadu. We report a case of subcutaneous human dirofilariasis of the eyelid in a 37 years old woman caused by *D. repens*.

Methods & Materials: A 37 year old female from urban Chennai with no co-morbidities presented with painless swelling of one month in the right eyelid which had a waxing/waning course. No other ocular or systemic features. No history of animal exposure. Ocular swelling was soft, cystic, non tender. Blood counts were normal with no eosinophilia. Provisional clinico-radiological diagnosis of epidermoid cyst or lacrimal adenitis was made and she underwent excision of lesion. Macroscopic examination revealed soft tissue grey-brown mass. Microscopic examination revealed eosinophils and fragments of adult nematode. Outer surface of the nematode's cuticle revealed longitudinal beaded ridges and transverse striations and was identified as *D. repens* which was confirmed by CDC. Microfilaraemia and filarial antigen test was negative. She was treated with ivermectin and diethylcarbamazine.

Results: Subcutaneous dirofilariasis is mostly caused by *D. repens* in Asia. Patients usually present with inflammatory subcutaneous masses containing increased numbers of eosinophils, which may or may not be tender. Ophthalmic involvement may be periorbital, subconjunctival, or intraocular. Eosinophilia is not usually present. Diagnosis of dirofilariasis in humans remains difficult as the symptoms exhibited by the patient are varying and nonspecific depending upon the location of worm. Identification of the worm in biopsy confirms diagnosis. Chemotherapeutic agents appear to be ineffective. Surgical removal of the worm is the treatment of choice.

Conclusion: A number of cases of human dirofilariasis from areas other than Kerala are being reported. Distribution of human cases of dirofilariasis seems to mirror the distribution of canine cases. Whether there a true increase in cases or were they earlier under reported, undiagnosed or unidentified due lack of awareness among the treating clinicians needs to be determined to know the actual prevalence.
Clinical profile and serological epidemiology of scrub typhus and spotted fever among hospitalized children at a tertiary hospital in South India

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Background: Scrub typhus, a re-emerging rickettsial disease caused by Orientia tsutsugamushi, is an important cause of febrile illness in the Asia-Pacific region. The present study was undertaken to evaluate the epidemiology, clinical profile and outcome of serologically confirmed scrub typhus and spotted fever cases among children admitted to a tertiary care hospital in Bangalore.

Methods & Materials: Hospitalized children aged <18 years, with clinical features suggestive of rickettsial disease were included prospectively between January 2010 to October 2012. Routine laboratory tests including Weil-Felix test was performed on all children. Specific ELISA was done to detect IgM and/or IgG antibodies for confirmation of scrub typhus and spotted fever.

Results: Of 103 children with clinical features suggestive of rickettsial illness, ELISA test confirmed 53 cases for scrub typhus, 23 cases for spotted fever group and 14 with both scrub typhus and spotted fever mixed infection. The mean age was 7.3 (±3.9) years and 44 (71.0%) were male. Majority of cases were from neighboring districts of Karnataka (50%), Andhra Pradesh (32.3%) and Tamil Nadu (17.7%). There was a clear seasonal trend; 53% of cases were seen soon after the rainy season during the months of August to November. Common clinical features included fever (100%) with average duration of 11 days, nausea and vomiting (44%) and rash (36%); eschar was rare. Anemia (63%), thrombocytopenia (52%), leukocytosis (51%) and elevated hepatic transaminases (61%) were also seen. Compared to the ELISA test, Weil-Felix test (OX-K titer of ≥1:80) had sensitivity and specificity of 88.7% and 43.9%, and positive and negative predictive value of 70.5% and 72%, respectively. Treatment with chloramphenicol or doxycycline was given to the majority of the children. Complications were seen in 42% (13% had multiple complications), and included meningoencephalitis (28%), shock (10%), retinal vasculitis (10%) and purpura fulminans (7%); all recovered with no deaths.

Conclusion: These findings suggest that the burden of rickettsial infection among children in India is high, with a substantially high complication rate. Rickettsial specific ELISA tests can help in early diagnosis and early institution of appropriate treatment that may prevent life-threatening complications.
Seroprevalence of Scrub typhus and coinfection with leptospirosis in Chennai, Tamil Nadu.

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Background: Scrub typhus is a rickettsial infection, caused by *Orientia tsutsugamushi*. It is a remerging infectious disease all over the world. In recent years, resurgence of Scrub typhus was noted in India especially in Tamil Nadu. Leptospirosis, a zoonotic infection caused by pathogenic spirochetes, *Leptospira interrogans* complex is prevalent worldwide with high incidences in tropical and subtropical countries. Both infections present as acute febrile illness. The present study was undertaken with the objective to estimate the Seroprevalence of Scrub typhus and leptospirosis in patients presenting with Pyrexia of Unknown Origin (PUO).

Methods & Materials: This prospective study was conducted in the Leptospirosis testing laboratory of the Department of Experimental Medicine from November 2014 to April 2015. All the serum samples from patients with PUO received at the laboratory were tested for Scrub Typhus Antibodies using Inbios International, Inc.Scrub Typhus Detect IgM ELISA System. The tests were performed and results interpreted as per the manufacturer’s instructions. Samples with OD value above 0.5 was considered as Reactive for Scrub Typhus. Microscopic Agglutination Test (MAT) was performed for diagnosis of Leptospirosis.

Results: A total of 354 serum samples were received during the study period. The age ranged from one to ninety years with mean 31.48 and SD 20.33. There were 40% males and 60% females. Among the 354 samples, 15% (30 males, 24 females) were positive for Scrub typhus by IgM ELISA. Highest seropositivity for Scrub typhus was observed in 0-10 followed by 21-30 years age groups. Out of the 354 sample, 43.7% were positive for Leptospirosis by MAT, with Canicola being the commonest serovar. Coinfection with Scrub typhus and leptospira was seen in 23 patients, Canicola was the commonest serovar of leptospira coinfected with scrub typhus.

Conclusion: In this study, Seroprevalence of leptospirosis and Scrub typhus was 43% and 15% respectively. Coinfection with Scrub typhus and Leptospirosis was observed in 23 patients. Morbidity and mortality is high in both infections particularly when diagnosis and treatment is delayed. Early diagnosis of scrub typhus and leptospirosis is essential since antibiotic therapy provides the greatest benefit when initiated early in the course of illness.
Pharmacophore modeling, database mining and biological evaluation to identify novel structurally diverse compounds as potential anti-Ebola drugs

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Background: Ebola virus disease (EVD), also known as Ebola hemorrhagic fever, is a rare and deadly disease caused by infection with one of the Ebola virus strains. It first appeared in 1976 and since then, there is no licensed treatment proven to counteract the virus but various immunological and drug therapies are under development. However, currently two potential candidates are undergoing evaluation. In view of the fact that virtual screening (VS) is an increasingly used method to guide the identification of novel hits from large chemical libraries.

Methods & Materials: Selection of Series with activity range span above 3.0 orders Hypothesis generation Using Hypogen module of Discovery Studio Followed by Model Validation and Virtual Screening Molecular docking studies to understand the type of molecular interactions within the active site of target using LibDocker/CDocker modules of Discovery Studio Biological Studies (In-Vitro/In-Vivo) to test the activity of the retrieved lead compounds

Results: We have tried to propose a research work which is structure and ligand based pharmacophore generation and chemical compound database mining (In-silico high throughput screening) followed by molecular docking to retrieve potent structurally diverse anti-Ebola drugs. The identified compounds will be further subjected to in-vivo studies. The identified compounds will be further subjected to in-vitro studies.

Conclusion: Reducing the research timeline in the drug discovery stage is a main concern worldwide. Various In silico techniques offering economical methods are now being used for the drug development. Molecular modelling is emerging as a popular methodology for drug design which aims at computer-aided techniques for the efficient identification and optimization of novel compounds with a required biological activity. Moreover, virtual screening is also a reliable and economical method as it helps in identification of new molecules which share its features and can thus exhibit a desired biological response. In view of the role of In-silico drug designing, the aim of the proposed research entitled “Pharmacophore Modeling, Database Mining and Biological Evaluation to Identify Novel Structurally Diverse compounds as Potential anti-Ebola drugs” is to elucidate the structural features responsible for anti-Ebola activity and to identify novel potential leads as anti-Ebola drugs.
West Nile Virus circulation and incrimination of mosquito vectors in Northeast India
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Background: West Nile virus (WNV) is a flavivirus transmitted by mosquitoes. The prevalence of WNV antibodies in Indian population has been known since 1952. Following phylogenetic analysis, Indian isolates were classified into Lineage 1 and 5. Since 2006, with the identification of WNV as an aetiology causing acute encephalitis syndrome (AES) in Northeast India, scattered cases have been recorded in Assam every year during the months of June to October. However, the mosquito species involved in transmission of WNV in this region requires detailed study.

Methods & Materials: We analysed human sera from hospitalised AES cases and mosquitoes sampled in four West Nile reporting areas during June 2014 - June 2015. Mosquitoes were caught using suction tube and flash light. A total of 970 pools from 37246 mosquitoes were based on uniformity of species, collection site and date. Clinical samples were tested for presence of WNV-specific IgM antibodies and confirmed by neutralizing antibody test for the acute and convalescent phase serum specimen. Both clinical samples and mosquito pools were also tested for the presence of WNV RNA using NS1 gene specific primers.

Results: Of the 222 AES acute case serum samples tested for the presence of WNV specific IgM antibodies, 48 were found positive. Six IgM positive samples demonstrated a fourfold rise in neutralizing antibody titre against WNV in convalescent sera. WNV RNA was detected in the cerebrospinal fluid of one human sample. Vector incrimination showed 14 pools (4- Culex vishnui, 1- Culex pseudovishnui, 2- Culex tritaeniorhynchus, 2- Culex quinquefasciatus, 1- Culex whitmorei and 4- Mansonia uniformis) to be positive for WNV RNA. Interestingly, 13 of the positive pools were collected during November - December 2014. Mosquito and human derived WNV sequence were similar to Indian Lineage 5 WNV isolates.

Conclusion: Our results provide molecular evidence for the persistence and maintenance of Lineage 5 WNV in Northeast India. This study confirms the role of Culex and Mansonia mosquito species in the transmission of WNV in Assam. Further studies are required to address WNV transmission and maintenance during winters for implementation of vector intervention strategies.
Molecular phylogenetics of Orientia tsutsugamushi strains circulating in Assam based on 56-kilodalton type-specific antigen gene

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Background: In India, scrub typhus (ST) is considered to be a re-emerging infectious disease. From northeast India, ST cases have recently been reported (2012) in Assam after a gap of 68 years. The causative organism, Orientia tsutsugamushi is known to be genetically and antigenically highly variable. Sequence analysis of 56-kilodalton (kda) type-specific antigen (TSA) gene has become an important tool for genetic characterization of Orientia. This variable gene sequence is useful for analysis of genetic diversity of Orientia isolates. Although re-emergence of ST has been reported, circulating strains and their origin have yet to be identified. In the present study, we determined the prevalent strains of O. tsutsugamushi circulating in Assam and their origin with respect to 56 kDa TSA gene.

Methods & Materials: 370 clinical serum samples from suspected scrub typhus and other unidentified fever cases were received from tertiary hospitals in Assam. Serological screening was done using Scrub Typhus Detect IgM ELISA (InBios International, Inc., USA). Positive and equivocal samples were subjected to PCR. Nested PCR was performed using primers specific for O. tsutsugamushi 56 kDa gene. Phylogenetic analysis was performed using MEGA 6 software.

Results: 19.4% sera (72/370) were found to be IgM positive and 6.4% (24/370) were equivocal. 13 of these samples were PCR positive for 56 kDa gene. Phylogenetic analysis of study strains showed variations in sequence homologies that formed 3 distinct clades that clustered with reference strains from: 1) India 2) Taiwan and 3) Thailand.

Conclusion: In summary, we have identified strains of O. tsutsugamushi circulating in Assam and established their evolutionary relationship with reference strains by analyzing a variable portion of 56 kDa gene. Understanding the evolution of the prevalent strains is important to understand the genetic differences which may help in planning control strategies as well as prophylactic measures including development of vaccines.
Uganda National Acute Febrile Illness Agent Detection Serosurvey 2004-2005

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Background: Due to their non-specific clinical presentation, acute febrile illnesses (AFI) are often diagnosed clinically as diseases known to be endemic to the region in which they are found. Uganda has been the site of multiple emerging-disease outbreaks, and there are several diseases that present with undifferentiated AFI, requiring further laboratory confirmation; however, limited laboratory capacity can impair timely diagnosis and public health interventions. This results in misdiagnosis and underreporting of emerging diseases of public health importance. The 2004-2005 Uganda National AFI Agent Detection Serosurvey (AFI serosurvey)—a retrospective investigation of seroprevalence of exposure to selected infectious agents—involves testing a subset of banked sera from the 2004-2005 Uganda HIV/AIDS Serobehavioural Survey (UHSBS). The AFI serosurvey is part of a multi-phase collaboration between Uganda Ministry of Health, Uganda Virus Research Institute (UVRI) and CDC-Atlanta/CDC-Uganda to investigate AFI in Uganda.

Methods & Materials: We selected a random 3097-sample subset from 19,656 UHSBS banked sera for inclusion in the AFI serosurvey; 2705 were ultimately analyzed after applying exclusion criteria. Data from laboratory testing were analyzed and mapped using SAS v9.3 and ArcGIS 10, respectively.

Results: Laboratory diagnostic testing results demonstrated: leptospirosis ELISA and microagglutination test (MAT) (10.4% weighted proportion, SE=1.2%), brucellosis MAT (0.3% weighted proportion, SE=0.1%), spotted fever group rickettsiae ELISA (56.7% weighted proportion, SE=1.4%) and typhus group rickettsiae ELISA (41.6% weighted proportion, SE=1.4%), malaria MSP119 ELISA (88.4% weighted proportion, SE=0.7%), orthopoxvirus IgG ELISA (13.5% weighted proportion, SE=0.8%), chikungunya IgM ELISA (31.1% weighted proportion, SE=1.0%), dengue IgM ELISA (1.0% weighted proportion, SE=0.2%) and IgG ELISA (0.7% weighted proportion, SE=0.2%). A specimen subset (n=198) was tested for melioidosis using indirect hemagglutination (IHA); 4.6% were seropositive.

Conclusion: Pre-existing national serosurveys can be a source of information on prevalence of AFI etiologic agents. This AFI serosurvey describes the distribution, regional risk, and interregional variability for selected diseases contributing to AFI across Uganda; it will inform prioritization of infectious disease surveillance and laboratory capacity-building activities. Results from this study combined with similarly obtained results from the ongoing testing from the 2011 Uganda AIDS Indicator Survey-based serosurvey will demonstrate changing seroprevalence patterns, allowing for evaluation of potential ecologic drivers for disease distribution variances.
Spotted fever group and typhus fever group rickettsiosis in South Western India

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\textbf{Background:} Rickettsial diseases are a group of zoonotic acute febrile illness transmitted to humans by vectors. They are classified into spotted fever group (SFG), typhus fever group (TG) and scrub typhus group (STG). STG remains the major cause of acute febrile illness requiring hospitalization in the tsutsugamushi triangle, however, the true picture of the SFG and TG rickettsiosis is not clear in India. Immunofluorescence assay (IFA) is the serological gold standard test for the diagnosis of rickettsial disease and was used to elucidate the disease burden in the Indian population requiring hospitalization.

\textbf{Methods & Materials:} Hospitalized patients with strong clinical suspicion towards rickettsial diseases other than ST were recruited from June 2012 and Jan 2015 at Kasturba Hospital, Manipal, India. Cases with significant titers by Weil-Felix test and/or those which were clinical diagnosed as rickettsial fever were further analyzed using IFA for confirmatory purposes. Slides were observed under a fluorescent microscope for fluorescence. Samples were screened at a dilution of 1:128 and positives, serially titrated to a dilution of 1024. Customized slides were supplied by the ARRL and standard IFA procedures followed.

\textbf{Results:} Out of the 1036 cases, 71 cases were suspected for rickettsial diseases other than ST. At a dilution of 1:128 dilution, 22 (2.1\%) cases were positive for SFG and 19 (1.8\%) cases for TG. Also, among these cases, 14 of the cases had shown significant titers for both SFG and TG. At endpoint dilution (ranging from 1:128-1:1024), there was cross reaction between strains among the SFG and to the TG. 18 cases were positive for R. australis, 16 for R. honei, 15 for R. conorii, 16 for R. africae, 15 for R. rickettsii, 11 for R. felis, 4 for R.prowazekii and 6 cases for R.typhi

\textbf{Conclusion:} SFG and TG rickettsiosis are prevalent in our part of the India. SFG is more prevalent than TG. Antigenic cross reactions between many strains were observed even at the end point dilutions and the exact nature of the disease causative strain could not be determined. Cultures and molecular confirmation of the causative agents will ultimately strengthen our findings.

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Background: Prior to the recent Ebola Virus Disease (EVD) outbreak, the pediatric age-group remained largely minimally affected or undetected/unreported. Given magnitude of this outbreak there has been a considerable proportion of children infected. This historical perspective may have limited inter-epidemic introduction of pediatric-specific treatment guidelines whereby clinical care was based on consultation of adult guidelines. There are limited studies analyzing pediatric clinical outcomes in EVD. In current study, we describe age-specific mortality and predictors of clinical outcomes of EVD in two districts where transmission happened in distinct phases and successive time periods.

Methods & Materials: We performed secondary data analysis on the national EVD outbreak database. Data was abstracted for Kailahun and Western districts for RT-PCR EBOV-positive and probable cases. We defined the study population as subjects aged ≤18 years in the database. Analysis was performed with STATA Version 12 package.

Ethical clearance was sought from Ministry of Health, Sierra Leone Research and Ethics committee.

Results: A total number of confirmed and probable cases in the two districts were 5,160 with male: female ratio 1:1, median age 27 years (IQR: 15-40). The majority, 4,497 (87.3%) were from the Western District. A total of 5130 (99.4%) presented alive to health facilities with fever and fatigue, 77% and 74.4% as the commonest presenting complaints respectively.

Pediatric observations accounted for 1,452 (28%) with majority (90%) from the Western District. The mean age was 8 years (SD±5.6) with males accounting for 48%. Overall pediatric mortality was 29% and highest in Western area with the most affected age groups being <2 and 2-5 years with 9.9% and 8.1% mortality respectively.

Probability to death in the first 6 days of the illness was highest for >2 years age-group (85%) followed by 2-5, 6-12 and >12<18 years; 74%, 50% and 27% respectively (log rank p value <0.0001).

Conclusion: The high pediatric mortality and deeper analysis of mortality findings in this study have not been documented before. Except the higher probability and shorter duration to death in this study, other findings are comparable with most of tropical diseases.

The belated introduction of pediatric clinical guidelines which coincided with EVD introduction in Western District did not reverse pediatric deaths.
Molecular identification of human plasmodium knowlesi infections in North Sumatera, Indonesia
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Background: Indonesia contributed 21% of malaria burden in Southeast Asia which caused by all Plasmodium species. However most reports predominate by Plasmodium falciparum and P. vivax due to routine diagnosis made by RDT and microscopy. The recognition of human P. knowlesi infection in Borneo for almost a decade ago was followed by similar identification of the species in other Southeast Asian countries. Until now, most diagnosis of P. knowlesi in human is made by molecular testing described by Singh et al. however this assay has been reported to have cross-reactivity to P. vivax infection. This study is part of drug efficacy study of P. falciparum malaria in North Sumatera. This study aims to investigate the distribution of Plasmodium species infecting human in the study areas, and to validate P. knowlesi-species specific diagnostic assay.

Methods & Materials: We carried out active and passive case detection in Batu Bara regency, Langkat regency and South Nias regency in North Sumatera province. We performed finger-prick sampling on 3635 participants for RDT, microscopy examination and filter paper dried blood spots. RDT were read on spot, thin and thick blood slides were stained in Giemsa and examined at the local health facility. Filter papers were transferred to LSHTM and DNA extraction were performed on all samples and screened for Plasmodium spp using species-specific nested PCR described by Snounou et al and newly designed P. knowlesi-specific primers.

Results: All Plasmodium species except P. ovale contributed in human malaria infections in three regencies in North Sumatera. Only P. falciparum and P. vivax were identified by microscopy. Both P. falciparum and P. vivax cases were distributed equally, while P. knowlesi contributed one-tenth of the total Plasmodium positive by PCR.

Conclusion: P. falciparum, P. vivax, P. malariae and P. knowlesi each contribute to human malaria infections in North Sumatera province. Improvement of diagnosis and increased awareness of P. knowlesi infection are needed owing to the high number of infections detected in this study.
Cronobacter sakazakii - An unrecognised food borne pathogen, India

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Background: Cronobacter sakazakii (formerly Enterobacter sakazakii) causes serious infections in infants with high mortality. Though contaminated powdered infant formula is the most common implicated vehicle, the organism has been isolated from other foods and environmental sources and infections have occurred among persons who did not consume or handle formula milk. There is significant underreporting of C. sakazakii infections in most countries and very little data is available from India. There is no information on human carriage, environmental sources and potential transmission routes of this pathogen from India. The aim of this study was to study the prevalence of this organism in North India (Haryana, Punjab and Chandigarh) and study the antibiotic resistance.

Methods & Materials: A wide range of samples (n=887) were collected from poultry farms, meat abattoirs (pork and mutton), dairy farms (pig stool, goat stool), human diarrhea stool samples and domestic environmental samples (water and soil) and processed for Cronobacter sakazakii. Presumptive C. sakazakii colonies were confirmed by MALDI-TOF. Antibiotic sensitivity was performed.

Results: Incidence of Cronobacter sakazakii was found to be 7.1% with individual incidence of 3.37% in humans, 9.4% in goat meat, 11.1% goat feces, 5.9% in pork, 7% pig stool, 6.7% in chicken stool, 11.5% environment. Of the 63 isolates, maximum resistance was observed against ampicillin (50.8%), followed by ciprofloxacin (20.6%) and then meropenem (17.2%). Carbapenem resistance was found in both animal food source and human strains.

Conclusion: Cronobacter sakazakii is part of fecal flora of humans as well as is carried by food production animals and is also present in domestic environment with potential risk of cross-contamination. The overall antibiotic resistance in human isolates was low in comparison to food animals and their fecal samples. A higher resistance in animal isolates could be due to rampant use of antibiotics as growth promoters in animals.
Crimean-Congo Hemorrhagic fever in former Soviet Union countries based on ProMED-RUS reports (2005-2015 years)

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Background: Russian ProMED-mail (ProMED-RUS) is one of ProMED’s regional networks that offers Russian language reports on emerging infections in 15 Former Soviet Union (FSU) countries. Several FSU countries have annual cases of Crimean-Congo hemorrhagic fever (CCHF). We wanted to review ProMED-RUS data to assess the epidemiology in the territory of FSU.

Methods & Materials: We used CCHF as the keyword to search reports in ProMED-RUS posted from 2005 to 2015. Comments by the moderators to complement the information provided in the outbreak itself were also reviewed.

Results: According to ProMED-RUS, CCHF were recorded in Russia, Kazakhstan, Georgia, Tajikistan, and Uzbekistan. The South of Russia (Stavropol, Rostov, Volgograd and Astrakhan regions, Kalmikiya, Ingushetiya, Dagestan, Karachaevo-Cherkessia) are endemic for CCHF. Between the years 2005 and 2015, 1,397 total cases, including 42 fatalities and a case fatality rate (CFR) of 4.2% were recorded. The highest numbers were registered between 2006 and 2008, which coincides with official Russian MOH statistics. Beginning in 2009, the incidence rate decreased, averaging 70 registered cases. In 2015 for the first time since 2009, the number of cases rose significantly and reached up to 139. ProMED-RUS reported about CCHF in Kazakhstan since 2008, with the highest number of cases (26) in 2009 in the southern regions - Jambyl and Kizilorda. Until 2015, 74 total cases and 16 fatal cases were registered with a CFR of 21.6% . In Tajikistan, ProMED-RUS reported about 5 cases in 2009 including 3 fatalities, CFR - 60%. ProMED-RUS published Georgia cases in 2012-2015: 2012 (1 case), 2013 (13 cases), 2014 (20 cases with 4 fatalities, CFR 20%) and this year 2 cases. In 2015 ProMED-RUS reported 13 CCHF cases in Uzbekistan within 2013-2015, including 10 fatal cases. CCHF starts in April, peaks between May and June, and decreases in August mostly due to tick bites. However, Kazakhstan (2009), Tajikistan (2009), and Russia (2011) registered 3 nosocomial clusters among healthcare workers due to inadequate infection control with 5, 7, and 9 cases respectively.

Conclusion: ProMED-RUS reports of outbreaks and comments from experts provide useful information for emerging infection case reporting, analysis, and comparison in the territory of FSU.
Clinical features and likely predictors of severity and fatality in dengue patients admitted to a tertiary care hospital in India

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Background: Early diagnosis of severe dengue is essential to reduce morbidity and mortality from the illness. The aim of our study was to evaluate clinical and laboratory indicators of severity and mortality in hospitalized dengue cases.

Methods & Materials: This was a 2-yr (2012-2014) retrospective study of all in-patients fulfilling the WHO diagnostic criteria of dengue. Clinical characteristics, laboratory parameters and treatment modalities were evaluated with respect to severity and mortality. Statistical analysis included c² test for categorical variables and Student’s t-test for continuous variables.

Results: Of the 550 ‘probable’ dengue inpatients over the study period, 126 were ‘severe dengue’, 364 were ‘dengue without warning signs’ and 60 were ‘dengue with warning signs (2009 WHO classification). Review of 21 fatalities, revealed that 48% (10/21) were between 40 to 60 years old. 11 of the fatal cases, had a Charlson comorbidity score > 3 (p=0.003). Bacterial coinfections had a significant association with mortality (7/21, p=0.002). Among the presenting symptoms, fever > 7 days, headache, rash, aches and pains, lethargy, restlessness and bleeding were significantly associated with fatality (p <0.05). Additionally hypoproteinemia, hypotension on admission correlated significantly with poor outcome. Older age (> 60 yrs) was significantly associated with severe dengue (p = 0.001) as was smoking and alcohol (p<0.05). Hemorrhagic manifestations, thrombocytopenia, pleural effusion and ascites had significant association with severe dengue (p<0.05). Detailed review of hemogram revealed that leucocytosis (WBC >10 K/cm³), lymphopenia (<20%) were associated with severity (p=0.0051) and fatality (p=0.000086). Hemoglobin less than 12 g/dl had a statistical correlation with severe dengue (p=0.0092). Hematocrit > 46 % was significantly associated with both severity (p=0.004) and fatality (p=0.045). Remarkably inflammatory markers like Neutrophil lymphocyte ratio of <5 on admission was significantly associated with severe dengue (p=0.003) and mortality (p=0). Admission RDW platelet ratio of < 0.12 and lymphocyte monocyte ratio <0.12 were both associated significantly with severity and fatality. Elevated urea and creatinine were associated significantly with severity and mortality(p<0.05).

Conclusion: Our observations highlight simple clinical and laboratory correlates of dengue severity and fatality. These could help clinicians recognise and aggressively manage the high risk patients to improve outcomes and morbidity.
GPI-anchored CCL28 as a strong mucosal immunostimulator with influenza VLPs

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Background: Influenza presents a major health problem, affecting hundreds of millions of people with high morbidity and mortality. Because of the importance of immunological regulations at mucosa, protection provided at the pathogen's entry site, and promising results with different infectious diseases at mucosa, in both clinical and experimental settings, it would be advantageous to study the mucosal adjuvant with influenza antigens.

Methods & Materials: The M1, HA/M1, HA/M1/GPI-CCL28 VLPs were prepared by co-infecting sf9 insect cells with rBVs expressing M1, HA and GPI-CCL28. The surface expression and in-vitro chemotactic activity towards CCR3+/CCR10+cells of GPI-CCL28 were checked using flowcytometry. All prepared VLPs were characterized and immunized in mice intranasally. The IgG/IgA responses were investigated in sera, tracheal, lung, and intestinal washes. We analyzed CD4+IFN-γ+cells for proliferation and CD8+CD107a+cells for cytolytic activities using FACS at spleen, lung, mediastinal lymph nodes, and peyer's patches. The Th1/Th2 cytokine and IgG/IgA secreting cells were estimated using ELISPOT while total concentration were checked by sandwich ELISA. Protective studies were evaluated in animals across distantly related subtypes.

Results: The HA/M1/GPI-CCL28 VLPs showed in-vitro chemotactic activity for CD3+/CCR3+/CCR10+cells and CD19+/CCR3+/CCR10+cells. The end point titer for IgG in sera and IgA in mucosal washes ranged between 51200-102400 and 6400-12800 respectively, in HA/M1/GPI-CCL28 VLPs, significantly (p<0.0001) higher (4-6 fold) than HA/M1 VLPs alone or HA/M1 VLPs with sCCL28 (soluble) with significantly higher IgG/IgA secreting cells. The IgG2a was observed higher than IgG1, indicating Th1 type of immune response. The HAI titer was significantly (p<0.001) higher (3-5 fold) in antibodies with HA/M1/GPI-CCL28 VLPs than other formulations. The HA/M1/GPI-CCL28 VLPs induced cell proliferation but not encouraged cytolytic activities. During cytokine estimations, a high IFN-γ and IL-2 with low IL-4 and TNF-α were observed. Protective efficacy was determined by challenging with A/Aichi/2/1968/H3N2 (homologous) and A/Philippines/2/1982/H3N2 (drifted) viruses and showed 100% and 80% survival in respective viruses, with no significant body weight lose, in the group of HA VLPs containing GPI-CCL28.

Conclusion: The GPI-CCL28 in influenza VLPs act as a strong immunostimulator at both systemic and mucosal sites when compared with influenza VLPs without CCL28, or influenza VLPs mixed with sCCL28, with significant protection in animals across distantly related subtypes.
Serological and molecular investigation of dengue, chikungunya and rift valley fever in febrile and non-febrile patients from northern Mozambique during Dengue outbreak, 2014

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Background: Arboviruses represent the most rapidly spreading mosquito-borne viruses worldwide. In Mozambique, arboviroses have been heavily neglected and for this reason they are never considered in the differential diagnosis of acute febrile illness. A recent dengue outbreak was reported in Pemba, situated in northern Mozambique in 2014, and during the outbreak more than half of dengue suspected cases had their dengue results negative, suggesting other causes of fever of unknown origin. The aim of the study was to investigate the circulation of dengue (DENV), chikungunya (CHIKV), west nile virus (WNV) and rift valley fever (RVF) in febrile and non-febrile patients in Pemba, during the outbreak of dengue in 2014.

Methods & Materials: A total of 398 individuals were identified, of which, 300 were non-febrile and 98 were dengue suspected cases (febrile) attending the outpatient appointment visit at Pemba Provincial Hospital, between March and April 2014 were enrolled in this study. Blood samples were collected from each participant and initially tested for dengue using IgM anti-dengue commercial ELISA and by real time PCR. All dengue negative samples were then tested for chikungunya, RVFV, WNV using the PCR and indirect immunofluorescence assay (IFA) for IgG detection.

Results: Of the 398 participants 37.7% (37/98) were DENV positive using PCR and all were negative for CHIKV, RVFV, WNV when tested by PCR. Among febrile patients, IgG antibodies against CHIKV were detected in 27.5% (27/98) of febrile participants by IFA, 10.2% (10/98) were dual positive for IgG anti-DENV and IgG anti-CHIKV and 3.06% (3/98) were positive for IgG anti-RVF. The percentage of positive results for IgG antibodies against DENV, CHIKV, RVFV among non-febrile participants were 26% (78/300), 45.3% (136/300) and 1% (3/300), respectively. No sample was positive for IgG anti-WNV antibody among non-febrile patients.

Conclusion: Our data provide evidence that DENV, CHIKV and RVF co-circulate in northern Mozambique, suggesting that not only DENV but CHIKV and RVFV, should be considered in the differential diagnosis of acute febrile syndrome in northern Mozambique.
Leishmania disease gap analysis study - Pakistan  
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**Background:** Pakistan is rated among one of the high Leishmania prevalent countries. The rising incidence of Leishmania in Pakistan are marked by poverty, poor healthcare, inadequate vector control, refugees, inadequate healthcare system and drug shortages. Disease is being reported from all over the country specially the remote underserved mountainous areas of Sindh, Khyber and Baluchistan provinces. To address this Pakistan OneHealth Alliance (POHA) in partnership with Ministry of Health Services (MOHS), and partners undertook, first ever, Gap Analysis Study in during April – October 2015. Plan was not only to identify gaps between diagnosis and management, but also, to help suggest a strategic plan to improve diagnosis, treatment, control and disease prevention.

**Methods & Materials:** A multidimensional approach was adopted to undertake this study. Primarily this was a cross sectional study based on questionnaire survey. Questionnaire were developed for multiple levels of healthcare system including community and patients. Data was captured from all over the country including some of the refugees camps.

**Results:** Cutaneous Leishmania (CL) is reportable under in National Health Information Systems. Clear inter provincial differences exist for disease reporting. 15 districts were found very high disease incidence. Unfortunately, for this disease there is neither an exclusive control program or integrated with some other disease. Only 38% of the facilities surveyed had adequate manpower and diagnostic facilities for the disease. There existed issues with availability of efficient detection and response system, staff training and availability of drugs. In about 50 % of facilities there existed severe drug shortage. Despite the high cost antimony injection was the main line of treatment for CL. This was followed by glucantine and cryotherapy. These drugs are neither manufactured.

**Conclusion:** Disease control demand greater political commitment by all stakeholders. Aggressive health education campaigns, inter sectoral collaboration, proper vector control, drug supply and preventive measures are desired to address this menace.
Synthetic DNA encoded antibody prophylaxis confers rapid protective immunity in vivo against Chikungunya virus infection


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**Background:** Chikungunya (CHIKV) disease is a serious emerging mosquito-transmitted viral infection responsible for epidemic outbreaks. Currently, no licensed vaccines or therapies are available against this virus, resulting in significant global spread with concurrent population morbidity.

**Methods & Materials:** This study describes a novel immune-based intervention that utilizes *in vivo* delivery of synthetic DNA plasmids encoding a monoclonal antibody (mAb) targeting the CHIKV envelope protein.

**Results:** Importantly, a single intramuscular injection with a DNA plasmid expressing an anti-CHIKV mAb produced antibodies in mice more rapidly than vaccination with CHIKV-Env-encoding DNA plasmids. DNA-mediated delivery of anti-CHIKV antibodies neutralized diverse clinical isolates *in vitro* and protected mice from lethal CHIKV challenge.

**Conclusion:** This study illustrates the ability of antibody-encoding plasmids to induce rapid protection against CHIKV highlighting the potential of this novel immunoprophylaxis and therapeutic applications platform for other emerging infectious pathogens.
Architectural suitability, designing achieving infection control and also the psychological comfort of the users: - Isolation centre (IC) for especially dangerous pathogens

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**Background:** Architectural suitability of a medical facility is very key to infection control, prevention and reduction of escalation of the infection in an individual. It contributes to prevention of spread of the infections to the staff and also to the community around the facility. These spaces should also accommodate the well-being of the users. Isolating the individual should not make them feel like prisoners but you cannot send them to the community. Therefore the space designed for this function should be well thought through to achieve the two contradictory statements. IC should be adequately located with adequate internal / room micro climate and building/ space layout. When they are poorly designed they may cause more harm than good because they may lead to infection of even the medical workers. **OBJECTIVES:** To examine the architectural suitability. To evaluate the suitability of the location, appropriateness of room micro-climate/ internal climate and the adequacy of the building/space layout plans, finishes and fixtures. And to establish factors that hinder architectural suitability.

**Methods & Materials:** I interviewed and discussed with the key sources of information like the users and administrators. I also physically visited and inspected some of the facilities at Entebbe and Mulago. I did desk study of the drawings and documentations for the existing designs.

**Results:** The existing Isolation Centres and medical facilities are not architecturally adequate thus a risk to infection and also they are unfriendly to the users thus resistances for staff there and also for patients to be taken there.

**Conclusion:** Most of the emerging epidemics of highly infectious diseases are actually from other nations and recently we have seen the effect of such epidemic on the capital city as the case in West Africa thus the need for a centre at the country’s main international access point and the capital city close to the national health excellence centre the national hospital. To improve the situation, the government should rectify the mistakes and infectious disease units should be created in all national hospitals and should be well designed so that cases can be well managed within the hospitals before transfer to the Isolation Facilities.
Cultural impact on the design for isolation centres for especially dangerous pathogens e.g. Ebola

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**Background:** From the recent Ebola outbreak in West Africa we realized that culture played a very significant role to the spread of the disease. The cultural activities for the dead and also the sick exposed many to Ebola. Isolation Centres that were initially set up did not put into consideration these cultural aspects thus the sick were hidden at their home preferring to die there with their families so they can have proper care in their last days and burial. There was no communication with their families and even no saying farewell to the dead. Male, female and children were put in the same space. **OBJECTIVES** To Design spaces within the Isolation Centre that allow for visiting of the patients and communication with their loved ones. Also come up with an innovation to have the relatives bid farewell to their loved ones. They cannot touch them, then let them see them and can also use an object to touch them like a stick or place their cultural objects. Allow for care from loved ones, one savior said they missed the "human hand."

**Methods & Materials:** I interviewed and discussed with the key sources of information like the users and administrators, I also physically visited and inspected some of the facilities at Entebbe and Mulago. I did desk study of the drawings and documentations for the existing designs. I took part in VHF and EDPs trainings.

**Results:** I came up with appropriate way to have the culture incorporated by appropriately designing visitors flow and area. Also the morgue has a facility where relatives can bid farewell to their loved ones. Also I proposed for better design of the isolation spaces to allow for easy waste disposal, cleaning and facilitation of treatment making it easier for staff to interact with the patients. Also I outlined designs clearly separating male, female and pediatrics.

**Conclusion:** For all space that are designed including isolation centres, it is important that yes infection prevention and control is number one consideration but without putting into consideration the culture of the area then people will run away and hide away from the facility.
Development and evaluation of a viral-specific random PCR and next-generation sequencing based assay for detection and sequencing of hand, foot, and mouth disease pathogens

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**Background:** Hand, foot, and mouth disease (HFMD) has become a major public health problem across the Asia-Pacific region, and is commonly caused by Enterovirus A, including enterovirus A71 (EV-A71) and coxsackievirus A (CV-A) 6, 10 and 16. Generating pathogen whole-genome sequences is essential for understanding their genetic diversity and phylodynamics. The frequent replacements among serotypes of Enterovirus A and a limited numbers of whole-genome sequences available in GenBank hinder the development of overlapping PCRs for whole-genome sequencing.

**Methods & Materials:** We developed and evaluated a viral-specific random PCR (rPCR) and next-generation sequencing based assay for sequence-independent whole-genome amplification and sequencing of HFMD pathogens. A total of 14 EV-A71/CV-A6/CV-A10/CV-A16 PCR positive rectal/throat swabs (Cp values: 20.9 – 33.3) were used for assay evaluation.

**Results:** Our viral-specific rPCR evidently outperformed the normal rPCR in terms of the total number of EV-A71 reads and the percentage of EV-A71 reads: 3% vs. 0.1% for the sample with Cp value of 30 and 6% vs. 0.91% for the sample with Cp value of 26, respectively. Additionally the assay could generate genome sequences with the percentages of coverage of 94%-100% of 4 different HFMD causing enteroviruses in 73% of the tested rectal/throat swabs, representing the first whole-genome sequences of CV-A6, CV-A10 and CV-A16 from Vietnam, and could assign correct serotyping results in 100% of the tested specimens. In all but one the obtained consensuses of two replicates from the same sample were 100% identical, suggesting that our assay is highly reproducible.

Phylogenetic analysis of the obtained sequences in this study suggested that the EV-A71 strains sampled in 2012 belonged to subgenogroup C4, whereas the viruses collected in 2013 belonged to subgenogroup B5. All CV-A16 sequences belonged to genogroup B1a, and showed a close relatedness to the viruses circulating in the Asia-Pacific region. Meanwhile the CV-A6 and CV-A10 strains were closely related to the corresponding HFMD-causing viruses from various parts of the world including Europe and Asia.

**Conclusion:** In conclusion, we have successfully developed a viral specific rPCR and next-generation sequencing based assay for sensitive detection and direct whole-genome sequencing of HFMD pathogens from clinical samples.
Could malaria re-emerge in Romania?

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Background: Romania was an endemic malaria country with as many as 300,000 new cases yearly and the eradication was completed in 1962. The permanent risk of malaria re-emergence in Romania maintains because of the simultaneous presence of the Anopheles maculipennis group vector species and imported malaria cases. The risk increased in the present conditions of climatic and other environmental changes.

Methods & Materials: The analysis of the historical and present data regarding the presence, abundance and distribution of different vector species in Romania in correlation with the environmental, social and economic conditions led to the evaluation of the risk evolution of malaria re-appearance and the main factors involved. The entomological data were integrated with earth observation data obtained by spatial technologies and mapped to put in evidence the stratification of the present risk areas of malaria re-emergence.

Results: The general level of malaria re-emergence risk varied in Romania. The risk was low after eradication linked to the low densities of vector populations because of the climatic conditions maintained in usual limits and intensive agriculture on large uniform areas. The risk gradually increased after 1990 linked to the high abundance of vector populations as before malaria eradication because of the global climate change and the land use change in Romania leading to the fragmentation of the habitat in the agricultural areas produced by the resuming to the traditional work on small private pieces of land. The present risk areas of malaria re-emergence are mapped. They generally overlap the former malaria stratification areas in Romania in accordance to the distribution of different vector species in the landscape. The new aspects are linked to the present environmental changes. Anopheles atroparvus, the main vector in Romania, has higher abundant populations and its distribution extended over all the risk areas. Anopheles daciae, possible malaria vector, has an extended distribution and higher densities than Anopheles messeae everywhere.

Conclusion: The malaria re-emergence risk maintains in Romania in conditions of the climate and other environmental changes. There is the need of the permanent surveillance of the factors influencing this risk to prevent and control malaria re-appearance.
Background: Different species of mosquitoes may have a role in the transmission of various diseases. The presence of more than one species in a habitat suggests that they share similar environmental conditions. Notwithstanding the importance of mosquitoes in the potential transmission of disease and intra and inter-species association, yet accurate data in relation to ecology and co-occurrence of mosquito species in the habitats is not available. Therefore, determining co-occurrence of this species in different habitats can be important to provide basic information in relation to vector control programs in the region. The present study focused on incidence of co-occurrence among mosquito species in Mazandaran Province, northern Iran.

Methods & Materials: Larvae collections were carried out from natural and artificial different habitats by standard dipping (350 cc) methods during May to December 2014, in 30 rural of 16 counties. Larvae collected from each larval habitats individually were preserved in test tubes containing lactophenol and sent to the laboratory for identification by valid key.

Results: Larvae were sampled from 120 habitats and identified by morphological keys. Sixteen species of mosquitoes were identified: An. claviger, An. hyrcanus, An. maculipennis, An. marteri, An. plumbeus, An. pseudopictus, Cx. pipiens, Cx. tritaeniorhynchus, Cx. torrentium, Cx. perexiguus, Cx. territans, Cx. mimeticus, Cx. hortensis, Cs. annulata, Cs. longiareolata, and Cs. morsitans. In total, larvae were seen in 1305 co-occurrences in natural and artificial oviposition sites during the study. The highest co-occurrence was observed associated with Cx. pipiens (630 occurrences, 48.27% of the total), An. maculipennis (87 occurrences, 6.66% of the total), respectively. Of these, Cx. pipiens was found in 123 occasions in related to Cx. torrentium which demonstrates the highest co-occurrences of the species. An. marteri (1, 0.07% of the total) and Cs. morsitans (2, 0.15% of the total) indicated the lowest occurrence in the province.

Conclusion: Cx. pipiens/Cx. torrentium shows highest co-occurrence in larval habitats in the province. Co-occurrence strengthen the common needs of these two species in the area which could indicate necessity further studies related to the bionomics Cx. pipiens/Cx. torrentium species, to provide adequate and affordable basic data for control programs in the future in the province.
Background: Arboviruses are transmitted by arthropods with humans becoming infected during blood feeding by infected mosquitoes, ticks and sandflies. Characterization of arbovirus circulation and transmission in industrialized countries has been well documented, but there are many knowledge gaps in developing nations. Entomological surveys conducted so far have indicated circulation of arboviruses of significant public health importance in Aedes, Anopheles and Culex species in vast populations in Kenya, suggesting the presence of competent vector systems.

Methods & Materials: The human involvement in the transmission cycle of these viruses has, however, not been demonstrated. This study sought to determine the circulation of a range of arboviruses including Chikungunya, Dengue, Sindbis, Sandfly Naples, Sandfly Sicilian, Uganda S, West Nile and Zika viruses in Ijara and Marigat Districts where vector surveillance has been done.

Results: A total of 351 patient serum samples were analyzed for presence of antibodies using IgG ELISA. Of these, 190 (54.2%) were female and 161 (45.8%) were female, with ages ranging between 1 and 73. These were hospital based patients who presented to the hospital with fever of unknown origin. The overall arbovirus percentage circulation among these patients was 53/351 (15.1%) with 7% (10/143) in Marigat and 21% (43/208) in Ijara. Of the positives, flaviviruses were 69%, alpha viruses 29.6% and bunyaviruses 1.4%. Uganda S virus was the highest in circulation at 10%, followed by West Nile virus 6%, Sindbis 5%, Dengue 2%, Chikungunya 1.1%, Sandfly Naples 0.2% respectively. Semliki-forest virus-specific antibodies were detected by plaque reduction neutralization test in 3/351 (0.85%) persons tested. Antibodies against Sandfly Sicilian and Zika viruses were not detected. This study constitutes the first detection of antibodies against Sandfly Naples virus in Kenya.

Conclusion: The study has demonstrated the presence of antibodies against selected arboviruses in the two sites amongst the human population. These findings will improve our understanding of the impact of arboviruses on public health in the region so that preventive actions and awareness among clinicians can be enhanced.
Serological evidence of henipavirus among horses and pigs in Zaria and environs, Kaduna State Nigeria


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Background: Henipavirus is an emerging, zoonotic and lethal RNA virus comprising Hendra virus (HeV) and Nipah virus (NiV), to which fruit bats are reservoir. Husbandry practices in Nigeria allow close contact between bat reservoir and animals susceptible to Henipavirus. This cross-sectional survey investigated antibodies reactive to Henipavirus sG antigen and associated risk factors in horses and pigs in Zaria, Nigeria.

Methods & Materials: Using convenience sampling, 200 horse sera (from four localities) and 310 pigs (from three localities) were screened by an indirect Henipavirus Enzyme-Linked immunosorbent assay (ELISA) (CSIRO, Australia). Structured questionnaires were employed with questions on the demographics and management of the animals. Data were analysed using SPSS-17.

Results: An over-all seroprevalence of 15.5% and 20% was obtained horses and pigs respectively. Seroprevalence was higher for horses managed intensively (21.1%); used for sports (25.5%); watered with pipe-borne water (17.9%); fed commercial feed (22.3%) and feed in the pen (17.6%). Also pigs managed intensively (58.1%); imported (69.5%); watered with pipe-borne water (31.3%); fed commercial feed (57.4%); feed in the pen (23.4%) and with a feed house (49.5%). Horses <5 years and pigs <6months had higher seroprevalences of 18.1% and 21.3% while the female horses and pigs had 19.8% and 22.8% respectively. Exotic horses and pigs revealed 25.5% and 55% while horses in Igabi and pigs in Giwa revealed: 24.7% and 70.2% seroprevalence respectively (P<0.05).

Conclusion: There is a suggestive evidence of Henipavirus in horses and pigs in Zaria, Nigeria with a huge public health implication. Local and exotic pigs and horses; pigs in Zaria and Sabon-Gari; horses in Zaria, Sabon-FGari and Kaduna North are associated with the seroprevalence of Henipavirus.
Serosurveillance of leptospirosis among paddy field workers with febrile illness from Western Maharashtra, India

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Background: Leptospirosis has the dubious distinction of being a zoonotic disease and an occupational disease. It is characterized by an acute febrile illness caused by spirochaetes of the pathogenic Leptospira interrogans. Leptospirosis occurs worldwide, in both rural and urban areas. The disease is most prevalent in temperate or tropical climates. It is an occupational hazard for rice and sugar-cane field workers, farmers, sewer workers, veterinarians, and dairy workers.

The aim of present study was to determine the sero-positivity for leptospirosis in paddy field workers with febrile illness and to identify the predominant serovars associated with this illness in Western India.

Methods & Materials: A cross sectional prospective study was conducted in 210 paddy field workers with acute febrile illness.

Blood samples were collected and subjected to culture (one drop of blood in 3ml EMJH media), IgM ELISA (Pan-Bio) and the microscopic agglutination test (MAT). MAT was done using a panel of 10 Leptospira serovars prevalent in this part of India.

All the samples were also tested for malaria, dengue, and typhoid fever to identify other common causes of febrile illness.

Results: IgM antibodies against leptospires were identified in 48 (22.85%) samples. MAT gave a positivity in 15 (7.14%) of the samples. Antibodies were predominantly seen against serovars Grippotyphosa 8 (53.33%), followed by Hebdomadis 4 (26.26%) and Lai like 3 (20%). Co-infections were seen with leptopsirosis in 3 patients. Two with typhoid fever, and one with Dengue.

Conclusion: Leptospirosis accounts for a little more than 1/5 th of the febrile illnesses in Western India. There could be a coinfection with other common etiologies of febrile illness such as typhoid and dengue and these must be looked for. The major prevailing serovars are Grippotyphosa and Hebdomadis, which must be kept in mind while developing a vaccine for this area.
Novel allelic profile of the clinical strains of burkholderia pseudomallei on multi locus sequence typing from India

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Background: Melioidosis is an emerging infectious disease caused by soil inhabitant gram negative bacterium Burkholderia pseudomallei. The disease which is endemic in Thailand and Northern Australia has shown an increasing trend in India in past few years. Epidemiological study of clinical isolates and their genetic relatedness to strains from neighboring south Asian countries remains undefined. The aim of the study was to analyze the molecular diversity of clinical B. pseudomallei isolates from southern India.

Methods & Materials: A total of 28 clinical strains of B. pseudomallei isolated from patients diagnosed with melioidosis at a tertiary care teaching hospital in southern India were included in the study. All isolated were subjected to Multilocus sequence typing (MLST) by amplification and sequencing of seven housekeeping genes. Sequences obtained from each locus were compared with the mlst database available on http://mlst.net. Further, sequence types (ST) were assigned for each isolate were tested for their genetic relatedness using e-burst analysis to the STs of isolates reported from neighboring countries of south Asia.

Results: Of the 28 isolates tested, 26 isolates had novel allelic profiles and 2 isolates matched with previously reported allelic profiles. Among the 26 isolates with novel allelic profiles, 15(53.6%) isolates were assigned as a new ST 1368 and the other 10 (38.4%) isolates were assigned STs 1369, 1370 (2), 1371(2), 1372, 1375(2), 1379 and 1380. One isolate had a novel allele in the gltB region. Two isolates which matched in their STs with previously reported isolates on the MLST database belonged to a single patient who presented initially with a systemic illness followed by a localized form of re-infection. Upon eburst analysis, we observed distinct molecular epidemiology of our isolates in comparison with STs reported from the neighboring countries of south Asia.

Conclusion: Predominance of a novel ST 1368 among our patients, which does not demonstrate genetic relatedness with other STs from neighboring countries of South Asia as well as from Australia was observed in the study. Further, our study findings underscore the high level of genetic diversity among clinical isolates of B. pseudomallei from coastal parts of south western India.
Modulations in HLA-DR expression in visceral leishmaniasis infection

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**Background:** HLA class II is a highly polymorphic cell surface glycoprotein, crucial for regulating host immune responses and thereby determining the disease outcome in visceral leishmaniasis by their ability to present the foreign antigens to CD4+ T lymphocytes and activating the T-cells. The complex regulation of HLA class II in different cellular compartments through class II transactivator gene (CIITA) determines the triggering and maintenance of immune responses and has vital role in determining the parasite clearance. The complex transcriptional regulation by CIITA is mediated upon its nuclear import by IFN-γ and hence regulating the expression of HLA-DR. This study tries to provide answer to the complex regulation of HLA-DR mediated via CIITA at the gene as well as the functional level in visceral leishmaniasis cases.

**Methods & Materials:** The study was performed on whole blood/PBMC and splenic aspirates from VL cases pre-/post-treatment and endemic control groups whole blood/PBMC. Gene(mRNA) expression was performed with the peripheral blood mononuclear cells(PBMC) and the splenic aspirates while the surface(protein) expression was performed with the whole blood and splenic aspirates from cases pre-/post-treatment.

**Results:** There was significantly lower expression of DRB1 (p=0.047) in active cases compared to healthy controls but there were no significant differences in DRB1 expression in active cases compared to paired cured cases in either PBMC (p=0.848) or splenic aspirates (p=0.628) while CIITA expression was higher at post-treatment as compared to pre-treated cases in splenic aspirates as well as the PBMC. At the cellular level, HLA-DR was significantly increased on CD14, CD16 and CD19 positive populations while CD4+ cells were having lowered kinetics of expression upon drug cure in splenic as well as the whole blood.

**Conclusion:** We found that the HLA-DR expression by cell types was similar in splenic aspirates as well as the whole blood assays but there were functional differences in the levels of HLA-DR expression at the gene as well as the protein level. The variable levels of expression of HLA-DR on macrophages and B-cells indicates for targeting these cell populations for modulating the immune responses by altering the antigen processing and presentation abilities hence designing the therapeutic/prophylactic options for VL.
Severe plasmodium vivax infections in children

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Background: Severe clinical cases exclusively associated with Plasmodium vivax are increasingly being reported worldwide with complications like renal failure, jaundice, acute respiratory distress syndrome, cerebral malaria, seizures, anemia, thrombocytopenia, pulmonary edema, splenic rupture and death. Recently vivax severity has been on the rise in India where P.vivax contributes in equal ratio with P.falciparum to the disease. Two main transporters studied with regard to chloroquine resistance (CQR) are chloroquine resistance transporter (pvcrt-o) and the multidrug resistance transporter (pvmdr1) orthologous to the pfcrt and pfmdr1 genes respectively. Even though these transporters are not established as molecular markers for CQR, they have a speculated role in CQR of P.vivax. Further, it has been demonstrated that the clinical severity in P.vivax could be associated with increased expression levels of pvcr-o and pvmdr1 genes. We report here two cases of vivax malaria- a severe and non-severe case, diagnosed and confirmed by microscopy, rapid diagnostic tests and 18S rRNA PCR assay.

Methods & Materials: Relative quantification was carried out to find the expression levels of five vir genes (vir 14-related, vir 12, vir 17-like, putative vir 14 and vir 10-related) and two P. vivax speculated drug resistance genes viz. P. vivax chloroquine resistance transporter (pvcrt-o) gene and P. vivax multidrug resistance transporter (pvmdr1) gene. These genes were selected on the basis of their in silico data and a previous understanding of their speculated role in the pathogenesis of P. vivax.

Results: Expression pvcrt-o and pvmdr1 genes and five vir genes (vir 14-related, vir 12, vir 17-like, putative vir 14 and vir 10-related), were measured simultaneously in these two cases for comparison. It was found that the expression levels of pvcrt-o and pvmdr1 genes and vir genes (vir 14-related, vir 12, vir 17-like and vir 10-related), was much higher in the severe vivax isolate as compared to the uncomplicated case. Putative vir14 gene was not expressed in the test and control isolates.

Conclusion: It brings to light how genes linked to the emerging CQR in P.vivax might impart virulence to vivax malaria making them excellent genetic markers for disease severity.
Implementing a novel community engagement system during a clinical trial of a candidate Ebola vaccine within an outbreak setting

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**Background:** Mistrust, suspicion and rumours have circulated around the purpose and motives behind recent Ebola vaccine clinical trials. EBOVAC-Salone is a community-based Phase II trial of the candidate Ad26.ZEBOV/MVA-BN-Filo prime-boost vaccine regimen against Ebola being conducted in Kambia, Sierra Leone. In this setting, we established a system for regular dialogue between trial staff and community members in order to build and sustain trust and to provide a mechanism to listen and respond promptly to rumours, concerns or misinformation in the community.

**Methods & Materials:** A local community liaison team engage with the community at levels. Strategies include one-to-one engagement with key stakeholders; public meetings with influential civil society and traditional leaders; house-to-house sensitisation, and use of traditional communication media such as radio. In addition, a social science team act as the ‘eyes and ears’ for the trial, listening to what individuals are saying about the study through both informal community observation and formal clinic exit interviews, in-depth interviews with participants and key stakeholders, and focus group discussions. The social science team provide daily feedback to the community liaison team on any rumours, concerns or misinformation circulating in the community, to inform prompt responses or clarifications. Furthermore, an external communications manager monitors national-level rumours and concerns and provides information on the trial at national and international level.

**Results:** Full engagement has occurred with both local leaders and the community. Enrolment in the trial has occurred successfully thus far, with trial participants voluntarily acting as ambassadors and encouraging others to volunteer. No disruption has occurred to the trial through rumours or misinformation thus far. Influential individuals in the community have assisted with disseminating messages and promptly addressing misconceptions.

**Conclusion:** Through a novel collaboration, we have formed an effective rapid response system for rumours and misinformation related to an Ebola vaccine trial during an outbreak. In addition, we have developed strong collaboration with the community through regular dialogue with stakeholders and trial participants. As a result, a community in rural Sierra Leone with no previous experience of medical research have developed a strong acceptance and understanding of the purpose of and processes involved in an Ebola vaccine trial.
Periodicity in the waxing and waning of Influenza A H1N1: A report from a tertiary care center in Chennai India

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Background: Influenza A H1N1 re emerged in 2009 causing a pandemic and continues to circulate worldwide seasonally. This study was undertaken to characterize Influenza A H1N1 in a tertiary care center over six years.

Methods & Materials: Throat swabs/nasopharyngeal samples were collected from patients who reported an influenza like illness (ILI) sent in viral transport medium in refrigerated condition to the laboratory. RNA was extracted from the clinical specimens. One step Real Time Reverse Transcriptase Polymerase Chain Reaction was performed (Ambion kit) using specific primers (INF A, universal SwineA, Swine H1 and RNaseP) and the Taqman probe (CDC protocol). Amplification was performed using Real time PCR (ABI 7900HT) system (reverse transcription for 30min at 50°C and initial activation for 10min at 95°C followed by 45 cycles of primer annealing and extension at 95°C for 15sec and 55°C for 30sec).

Results: A total of 2382 samples were analyzed, n= 480(20.15%) tested positive for novel Influenza A H1N1 and n=102(4.28%) tested positive for seasonal influenza A. The positivity rate was 46.08% in 2009, fell from 15.02% (2010), to 1% by 2011; was 22% in 2012 just 1.86% in 2013, 7.096% in 2014 and is currently 44.2% as of Oct 2015. The age of patients ranged from 10 days to >81 years. Influenza Ah1n1 accounted for 25.35% of ILI in children (0-18 years). The rate of positivity in adults was found to be similar across age groups of 21-60 years (range 19.7%-21.1%) and declined to 13.5% in greater than 61 years of age. Cough (84.2%) was the predominant symptom, followed by fever (83.75%), breathlessness (55.2%), body ache (54.8%), vomiting (21.4%), diarrhoea (9.58%), 4.4% gave history of travel abroad. Based on the duration of illness the rate of positivity was found to be 78.54% from day one to day 7 of ILI. However 5.62% were positive between day 10 to 30.

Conclusion: There is a cyclical occurrence in the seasonality of Influenza H1N1. Vulnerable groups such as pregnant and lactating mothers may require targeted intervention.
Clinical features, cytokine profiles and immune response in children with severe hand foot and mouth disease in Vietnam

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**Background:** Hand, foot and mouth disease (HFMD) is an emerging infection in Asia. Neurological complication and fatality are typically associated with enterovirus A71 (EV-A71). Intravenous immunoglobulin (IVIg) is widely used in the treatment of severe cases, albeit without clinical evidence. This study characterized clinical features, virology, cytokine profiles and immune responses in severe HFMD children receiving IVIg.

**Methods & Materials:** A prospective study was conducted in the ICU of Children’s Hospital 2 (Ho Chi Minh City, Vietnam) from June to September 2012 enrolled HFMD children with clinical indication to have CSF taken. Clinical data, diagnostic throat/rectal swabs, CSF after IVIg, plasma before and after IVIg and at discharge were collected. Plasma cytokines/chemokines and antibodies against common HFMD associated viruses were assessed before and after IVIg and at discharge. Cytokines/chemokines were assessed in CSF.

**Results:** Thirty patients were enrolled (grade 2b, n=15, grade 3, 13 and grade 4, 2). Twenty-five patients recovered, 2 died and 3 with unknown outcome due to withdrawing. Clinical characteristics improved within 48 hours of admission in grade 2b and after 72 hours in grade 3 patients, coinciding with 1st and 2nd doses of IVIg administration. EV-A71 was detected in swabs of 25 patients and in CSF of 1, and other EVs in swabs of 3. The level of 10 cytokines/chemokines in CSF was comparable between patients receiving one and two IVIg doses, which were also unchanged before and after IVIg in plasma. At discharge, plasma IL-1β, IL-6, IL-10, GM-CSF and IFN-γ were significantly decreased (Figure1). CSF IL-1β, IL-4, IL-10 and TNF-α were significantly lower as compared to that in plasma after IVIg. In contrast, CSF IL-6 was significantly higher than that in plasma after the 2nd IVIg (P <0.05) (Figure2). Before IVIg, plasma neutralizing antibody levels against EV-A71 subgenogroup C4 and B5 were high but that against CV-A10/A16/12 were low (Figure3). These titers increased after IVIg except for that against CV-A12.

**Conclusion:** We have described the clinical characteristics, immunological profile and outcome of 30 severe HFMD children with IVIg administration, which is informative for disease management. Cross-neutralization data from this study is important for vaccine design and development in the future.
Atypical presentation of epidemic typhus in South India: A case report
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Background: Epidemic typhus is due to R. prowazekii. In India the endemic spot is Kashmir. Infection is transmitted when the contaminated louse faces is rubbed through the minute abrasions caused by scratching. Occasionally, infection may also be transmitted by aerosols of dried louse faces through inhalation or through the conjunctiva. Incubation period is 5 - 15 days. They infect the vascular endothelium and reticuloendothelial cells with 40% case fatality. A characteristic rash sparing the face, palms and soles. Towards the second week, the patient becomes stuporous and delirious. Thrombocytopenia is observed in more than half of the patients.

Methods & Materials: A 17 years old male patient, residing in hostel, complained of high grade fever since 9 days and after 4 days of fever, rashes appeared first on trunk which spread over limbs but sparing face, palm and sole and consequently changed into purpura fulminans. On day 6th patient had syncope with seizure. On day 9th patient was admitted in ICU because of altered sensorium with left facial paralysis without neck rigidity. Immediately empirical treatment was started with cephaperazone-sulbactum, doxycycline and acyclovir and de-escalated subsequently. Investigation showed Hb 13.6 gm/dl, WBC 14900 /mm3 (Neutrophil 79%) and platelet 26000/mm3. CSF and other blood examination done were normal. Weil-Felix test was positive (by tube agglutination, to proteus antigen OX19 (1:640); OXK (1:640); OX2 (negative)). CT and further MRI brain were normal.

Results: The case appears atypical because suspected encephalitis resolved fast in one day without any sequel. Patient had high grade fever till day 17, which responded to 12 days of doxycycline. Initial presentation of case was like acute stroke and review of literature also sparsely reported this but investigations did not support this. Suspected neurorickettsioses disappeared rapidly, is also very atypical in pathogenesis of vasculitis. Prolonged high grade fever till day 17, was investigated for malaria and tuberculous meningitis. Weil-Felix Test report was high with equal titre in OX19 & OXK which is also atypical.

Conclusion: For rare organisms, clinical presentation and investigation reports can be vague. Lack of facility to confirm rickettsioses is the biggest limitation. In current scenario Rickettsioses is diagnosed by relevant clinical findings and Weil-Felix test positivity.
Real-Time PCR studies regarding the borrelia burgdorferi, francisella tularensis, tick borne encephalitis virus (TBEv) and crimean congo hemorrhagic fever virus (CCHFv) occurrence in the Romanian ticks

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Background: Our studies undertaken between 2006-2015 have shown that the most frequent species of ticks in Romania is Ixodes ricinus. It was found that I. ricinus is the main vector in Romania for the Borrelia burgdorferi s.l. and for the TBEv, but no data were available for Francisella tularensis and CCHFv infectious agents occurrence into I. ricinus and Hyalomma sp.

Three Romanian counties were selected as ticks sampling sites (Sibiu, Tulcea and Giurgiu), with this occasion we collected ticks from the vegetation and from the livestock and reptile fauna. Among the reptiles (Tulcea county), Testudo graeca ibera (TGI) is a well represented species. Samples of ectoparasites obtained from TGI and livestock collected during the years: 2006-2007 and 2014 - 2015 (April-June) showed that the majority of ticks are represented by Hyalomma.aegyptium and H. marginatum.

Over 400 I. ricinus and Hyalomma sp. ticks were collected and analyzed by Real-Time PCR methods (including the new TicKitqPCR detection concept; project funded by the MEN-UEFISCDI PN II „Partnerships in priority areas” program, National Research Grant No. 295/2014") that give us results on Borrelia burgdorferi, Francisella tularensis, TBEv and CCHFv (BFTC) presence in the vectors.

Methods & Materials: Total RNA and DNA were extracted and analyzed by in house real-time PCR reagents (included in the new TickKitqPCR detection concept) and 2 commercial kits for the BFTC detection in the I. ricinus and Hyalomma sp. pools.

Results: Specific DNAs from B. burgdorferi sl. were detected (Fla B gene target) in 20% of I. ricinus ticks and specific DNAs for F. tularensis were detected (IS Ftu2 genomic isertion-like element) in 2% of the same vector species.

Specific RNAs from TBEv were detected (3’ UTR-genomic region) in < 1% of I. ricinus pools.

No specific CCHFv RNAs were detected (S –genomic region) among the Hyalomma sp. pools.

Conclusion: The results strengthen the concern that already exists in Romania, for the enhancement the surveillance and the control measures for the tick populations but also for the means of active information of the human population about the danger of the diseases transmitted by ticks in some risk areas.
Background: We are transforming the field of infectious disease diagnostics with the development of the Sample Prep for Infectious Disease Recognition With EDGE Bioinformatics (SPIDR-WEB). SPIDR-WEB is a sample-to-result biotechnology platform that enables efficient use of next generation sequencing (NGS) for pathogen detection in clinical samples. NGS has become a powerful tool for detection and characterization of both known and emerging pathogens. The main advantage of NGS is its non-biased approach that identifies all organisms in a sample. This is in contrast to traditional molecular assays that force us to look for a set of specific pathogens. In most clinical samples, the relative abundance of pathogen nucleic acids (DNA or RNA) is vanishingly small. Therefore, vast amounts of sequence data must be generated and analyzed to identify rare pathogen sequences. SPIDR-WEB is a sample-to-result process that relies on efficient laboratory and in silico steps.

Methods & Materials: Clinical samples mostly comprise non-informative host RNAs or abundant housekeeping gene transcripts. SPIDR-WEB incorporates removal of non-informative RNAs (RNR), thereby enriching all other RNAs, including those from pathogens. This step enables either higher sensitivity and specificity, or less expensive and faster sequencing. Our custom EDGE bioinformatics data analysis platform provides rapid read classification at all taxonomic levels, and reliably detects all organisms present in a sample. EDGE is an efficient process, as it uses databases with pre-computed signatures, instead of aligning sequencing reads to the entire Genbank. In addition to RNR and EDGE, SPIDR-WEB includes robust, inexpensive and rapid sample lysis, RNA extraction, and library preparation steps.

Results: We will describe SPIDR-WEB technology and show clinically-relevant results obtained from human blood, stool, respiratory, and other sample types.

Conclusion: We are implementing SPIDR-WEB in both research and clinical settings to support a multitude of applications, such as discovery of novel mechanisms and biomarkers, study host-pathogen interactions, improve vaccines and therapeutics, and complement current diagnostic tools and help improve their utility.
Yellow fever threat to Asia: A model national contingency plan

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**Background:** In December 2015, the Hong Kong Standard reported that its hospitals were ready to handle dengue and yellow fever outbreaks. There has never been a case of yellow fever in HK, or anywhere else in Asia. Nobody knows why not. But the risk is there. HK could lead Asia by preparing a contingency plan. Why? Because when YF arrives in HK everyone will think it is only jaundice or dengue.

**Methods & Materials:** Do the medical labs have the reagents to distinguish yellow fever from closely related dengue? Is there a stockpile of the vaccine? There is no treatment. Yellow fever kills 20% of unvaccinated people, the vaccine is only fully effective 10 days after the shot, and infants under 9 months and seniors over 65 should not have it because of possible side effects. Is there a stockpile of mosquito control chemicals, ground & air fogging equipment and technicians who know how to calibrate the spray and get themselves tested for the side effects of their exposure to the spray?

**Results:** A PowerPoint slide show of how to prepare a contingency plan is available from WHO/SEARO New Delhi.

**Conclusion:** We urge all Asian countries at risk-- particularly India & southern China – to draft a contingency plan as soon as possible. This also applies to every country in Asia that has dengue, because the same mosquitoes that transmit dengue can transmit YF from an imported case. This is to avoid falling into the same unprepared situation that West Africa experienced with Ebola.

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Eating raw liver, a potential risk factor of Crimean-Congo hemorrhagic fever (CCHF) occurrence in high-risk occupations in Nur County, Northern Iran

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Background: Eating raw lamb or beef liver is of interest to some people such as livestock farmers, shepherds, butchers and chefs. This high risk practice can cause gastrointestinal problems such as parasitic diseases and microbial infection of the liver tissue and also causes transmission of Crimean-Congo Hemorrhagic Fever (CCHF) to humans. CCHF cases occurrence in a butcher and a farmer in Nur County, raised the necessity to check the background of high risk behavior related to the consumption of raw liver in individuals who work in livestock and meat industry. This can help prevention of the disease in high-risk occupation groups.

Methods & Materials: In 2012, a cross-sectional study on 314 people, including livestock farmers, butchers, abattoir workers, chefs and veterinary staff was undertaken in three district of the Nur County. The practice of eating raw liver in different high risk occupation groups was recorded in a standard questionnaire through interview. The relevance of this high risk behavior with qualitative variables by Chi-square test and binary logistic regression were analyzed at the significant level of 0.05.

Results: The odds ratio (OR) of raw liver eating was significantly higher in livestock farmers and animal keepers than other occupations (OR = 15.27, CI: 2.04-114.32), in the mountains than plains and woodland areas (OR = 3.47, CI: 1.66-7.29) and in Baladeh district than other districts including Central and Chamestan District of the County (OR = 2.49, CI: 1.14-5.42), respectively. Additionally, consumption of raw liver in 30-39 year old age group was higher than other age groups (OR = 2.06, CI: 0.81-5.22), it was higher in rural population than urban residents (OR = 1.79, CI: 0.72-4.46). The prevalence of this behavior in mountainous areas and Baladeh District of Nur County may be explained by the high frequency of traditional animal husbandry and unsafe slaughtering, low literacy levels, difficulty in implementing policies on health education in rural areas away from the County.

Conclusion: People attempt to eat raw liver in high risk occupational groups can increase the risk of CCHF and even its epidemic in the region.
Outbreak of prototheca wickerhamii algaemia and sepsis in a tertiary care chemotherapy oncology unit

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**Background:** *Prototheca* is an emerging, rare, opportunistic, pathogenic, achlorophyllous green alga known to cause Protothecosis which is a zoonotic disease. Earlier interpreted as contaminants in blood and faeces, *Prototheca* is expanding its pathogenicity and host range. An outbreak of Protothecosis by *Prototheca wickerhamii* in a tertiary care chemotherapy oncology unit is being discussed.

**Methods & Materials:** All patients detected to have algaemia were operationally included in the case definition. Clinicodemographic profile, diagnosis, duration of stay, treatment protocol and neutrophil count were correlated. After isolation on sheep blood and Sabouraud’s agars, urease, Germ tube formation and automated identification through VITEK 2 (bioMérieux, France) were attempted. Colony characteristics, micromorphology, substrate utilization and antifungal susceptibility were interpreted. All patients were initiated on liposomal amphotericin B (5 mg/kg body weight/day). Fecal cultures of affected patients, environmental surveillance and healthcare staff were screened while continuing surveillance for one year post outbreak.

**Results:** The outbreak lasted approximately 50 days during which the average occupancy was 26 patients (86.67%) and mean hospital stay was 60 days. Mean age of affected patients was 37 ± 10.74 years with male: female:: 5: 1. Mean neutrophil count in affected patients was 150 per dl. The attack rate was 7.69. *Prototheca wickerhamii* was isolated on sheep blood and Sabouraud’s agars as yeast-like colonies having Gram positive 3-11 µ non-capsulated spherical yeast-like cells without budding and pseudohyphae. All isolates were negative for urease and Germ tube formation. VITEK 2 compact provided 99% identification probability. MICs in µg/ml for Amphotericin B and Voriconazole were 0.5 and 2 respectively. All isolates were similar for biochemical reactions and susceptibility patterns. All patients responded to liposomal amphotericin B. One patient detected to have algaemia went into sepsis with serum procalcitonin levels between 2-4 ng/ml with subsequent fatal outcome under intensive care. Surveillance studies were not contributory.

**Conclusion:** Immunocompromised neutropenic patients having Protothecosis may not manifest clinical features leaving detection to intuitive clinical acumen. Outbreaks are difficult to detect and control as incubation period is variable. Such hospital outbreaks re-emphasize the need to strengthen hospital and laboratory based surveillance to ensure adequate preparedness, rapid detection and response to outbreaks.
Serotyping of invasive S. pneumoniae in adults, more than fifty years old, at a tertiary care center

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Background: The vast majority of the epidemiological data on the pneumococcal disease is largely available in the pediatric population; however there is limited data in adults from India. In the present study we evaluated antimicrobial susceptibilities testing (AST) and serotypes of pneumococcal isolates in adult population.

Methods & Materials: The study was a hospital based prospective observational study. Fifty consecutive S. pneumoniae isolates from patients ≥ 50 years with community acquired pneumococcal infections from various sites (invasive and non invasive) were included in the study. We performed antimicrobial susceptibility testing (AST) by disk diffusion and MIC testing by E-test and serotyping by Quellung anti-capsular serotyping on these isolates.

Results: AST showed 100% sensitivity to penicillin, ceftriaxone and vancomycin. Levofloxacin, co-trimoxazole, erythromycin and clindamycin showed sensitivity of 84%, 26%, 90% and 94%, respectively. There was low prevalence of multi-drug-resistant S. pneumoniae (6%) observed in our study. Common serotypes identified were: 19A: (14%), 8: (10%), 19F: (8%); 3 and 9N: (6% each). Non-vaccine serotypes (NVS) comprised 30% of the isolates with no predominant serotypes. PCV7, PCV10, PCV13 and PCV23 vaccine coverage was observed as 16%, 24%, and 66% respectively. The invasive serotypes comprised 38% of the total isolates and 42.1% of these isolates were NVS.

Conclusion: Our study shows nil resistance of S. pneumoniae to penicillin and a low prevalence of MDR. On the contrary there was a high prevalence of resistance to co-trimoxazole and levofloxacin as compared to other asian countries. More importantly our study showed emergence of 19A serotype as the predominant serotype. There was also a poor coverage of currently available PCV13 (24%) and PPV23 (66%) pneumococcal vaccines in adults, and more importantly among invasive serotypes. In summary, our findings show that the current vaccines recommended for adult immunization program has modest efficacy in Indian population, especially in invasive infections. However PPV 23 vaccine appears to be the best option as on today but Indian data may throw challenges for the PPV23 pneumococcal vaccine. Therefore, continuous surveillance from different parts of India is needed for prudent use of S. pneumoniae vaccination.
Trying to understand infections in transplant patients in a private hospital in Buenos Aires, Argentina.

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Background: Since the first renal transplantation performed in Boston in 1954, solid organ transplantation became a common strategy against end-stage diseases. The German Hospital in Buenos Aires performed its first renal transplantation in 2000 and since then practice grew including also liver and heart transplantations. After 15 years it is time to evaluate the current infectious complications, aiming to discover useful variables to work on.

Methods & Materials: This analysis is a retrospective observational study, for which we have reviewed the medical records of all patients undergoing transplantation surgeries from 1-Jan-2014 to 31-Dec-2014. On an excel sheet we have analyzed information such as: age, gender, underlying disease, type of immune suppression, time of onset of the infectious event and type and source of microorganisms involved.

Results: Forty-six patients were transplanted during 2014, 33 (71.7%) of them had at least one infectious event. Median age was 55 (8-78, 70% between 31-65 years), 74% males. There was no difference between infected and not-infected regarding these 2 points. Organs transplanted: 2 hearts, 21 kidneys and 23 livers. Percentage of infections was similar in the different groups. Twenty-seven (33%) of infectious events were due to urinary tract infections, 19 of them in renal transplants (70%, p=0.02). CMV-reactivation was seen in 12 cases, 9 (75%) of them in liver-transplantations. Primary bacteremia was in third place (9, 13%) and surgical site infection in fourth (7, 10%). Low numbers prevent from calculating rates. Most of the infections (88%) showed up during the first 3 months, only 1 (3%) after 6 months. There was a wide range of microorganisms involved, 68% bacteria (70% GNB), 19% virus, 95 fungus and 4% TB. Regarding the storage fluid, 24% presented bacterial growth. There wasn’t an increase incidence of infectious events in those in which the storage fluid was contaminated.

Conclusion: Urinary tract infection was the main complication as literature mentions. Surgical site infections were not prevalent in a particular group, which rules out inappropriate surgical technique. The variety of microorganisms involved rules out a common source. CMV prophylaxis strategy in hepatic transplant patients has to be reviewed.
Post renal transplant infections: Single center experience from Nigeria.

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\textsuperscript{1}Bayero University Kano, Kano, Kano, Nigeria, \textsuperscript{2}Bayero university Kano, Kano, Nigeria, \textsuperscript{3}Aminu Kano Teaching Hospital, Kano, Nigeria, \textsuperscript{4}Bayero University Kano, Kano, Nigeria

\textbf{Background:} Infections are the leading cause of hospitalization and mortality in transplant recipients. Nigeria has a growing number of renal transplant recipients. The aim of this study was to determine the pattern of infections in renal allograft recipients in one of the major renal transplant centers in Nigeria.

\textbf{Methods & Materials:} All case records of renal allograft recipients on follow up were retrieved. Those that had infection at any time after transplantation were selected. Demographic and clinical information were collected and analysed.

\textbf{Results:} Thirty three case records were analysed out of which 24/33(72.7\%) were males, with the mean age of 42.3 years (±7.38). The median duration of developing infection post-transplant was 270 days (Range 2 – 2190). Most of the infection occurred after 6month 15/33(45.5\%). Urinary tract infection was the commonest infection 13/33(39.4\%), followed by pneumonia 12(33.3\%). There were two cases each (5.6\%) of tuberculosis and non-typhoidal salmonella septicemia and one case (2.8\%) of CMV colitis. Majority 6/9 (66.7\%) of the pneumonia cases was gram negative pneumonia with \textit{P. aeruginosa} been the commonest isolates 3/9(33.3\%). Among those with UTI, \textit{E.coli}, and \textit{Klebsiella spp} were isolated with equal proportion 3/13(23.1\%) while \textit{Enterococcus faecalis} was the commonest isolate 4/13(30.8\%). Among the tested isolates 61.5\% and 50\% of gram negative pathogens and \textit{S. aureus} respectively were resistant to co-trimoxazole, while all the 2 isolated non-typhoidal salmonella were MDR. Overall mortality was 10/33(30.3\%) out of which 5/10(50\%) was due to pneumonia.

\textbf{Conclusion:} There is need to strengthen infection surveillance among post-transplant patients in Nigeria. Infection with MDR pathogen is common and the outcome co-trimoxazole prophylaxis in renal transplantation in our center need to be reviewed.
Infectious complications post liver transplant in a tertiary hospital
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Apollo Hospital, Hyderabad, India

Background: Infection is a leading cause of morbidity and mortality in liver transplant recipients, with more than two-thirds of liver transplant recipients having infections in the first year after transplantation. The aim of the study is to evaluate the incidence, type, pathogen, and timing of infections post orthotopic liver transplant recipients in the Indian scenario. The study will help formulate strategies for prophylaxis and treatment of common infections seen in the intermediate and late post-transplant period.

Methods & Materials: This is a retrospective, single centre, observational study. Records of 64 consecutive liver transplant recipients (live and deceased donor) between March 6, 2014 and October 15, 2015, were analyzed. Infectious episodes were classified based on the time after transplant:
* Early – less than 30 days
* Intermediate: 1-6 months
* Delayed: After 6 months

Results: 23 (35.9%) patients had infections post transplant and 4 (6.25%) died due to its complications. Mean follow-up period was 9.75 months (range 0.5 months to 19 months).

Table 1. Time

<table>
<thead>
<tr>
<th>TIME</th>
<th>Number of infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 30 days</td>
<td>Total 20 (66.67%)</td>
</tr>
<tr>
<td></td>
<td>Bacterial 9</td>
</tr>
<tr>
<td></td>
<td>Viral 4</td>
</tr>
<tr>
<td></td>
<td>Fungal 4</td>
</tr>
<tr>
<td></td>
<td>Unknown 3</td>
</tr>
<tr>
<td>1-6 months</td>
<td>Total 8 (26.67%)</td>
</tr>
<tr>
<td></td>
<td>Viral 5</td>
</tr>
<tr>
<td></td>
<td>Bacterial 1</td>
</tr>
<tr>
<td></td>
<td>Unknown 2</td>
</tr>
<tr>
<td>After 6 months</td>
<td>Total 2 (6.67%)</td>
</tr>
<tr>
<td></td>
<td>Viral 2</td>
</tr>
</tbody>
</table>

Table 2. Pathogen

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number of Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial (Total)</td>
<td>10 (33.3%)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>6</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>1</td>
</tr>
<tr>
<td>E.Coli</td>
<td>2</td>
</tr>
<tr>
<td>C.Difficile</td>
<td>1</td>
</tr>
<tr>
<td>Viral (Total)</td>
<td>11 (36.67%)</td>
</tr>
<tr>
<td>CMV</td>
<td>7</td>
</tr>
<tr>
<td>Varicella Zoster</td>
<td>14 (46.67%)</td>
</tr>
<tr>
<td>Fungal (Total)</td>
<td>4 (13.33%)</td>
</tr>
<tr>
<td>Candida</td>
<td>2</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>2</td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (16.67%)</td>
</tr>
</tbody>
</table>

Table 3. Site

<table>
<thead>
<tr>
<th>BACTERIAL</th>
<th>Number of infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Stream</td>
<td>5</td>
</tr>
<tr>
<td>Surgical Site</td>
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<tr>
<td>Respiratory Tract</td>
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</tr>
<tr>
<td>Genito-Urinary Tract</td>
<td>2</td>
</tr>
<tr>
<td>Intra-Abdominal</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VIRAL</th>
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<tr>
<td>Blood Stream</td>
<td>7</td>
</tr>
<tr>
<td>Skin and Soft Tissue</td>
<td>4</td>
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<table>
<thead>
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<th>FUNGAL</th>
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<tr>
<td>Blood Stream</td>
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<tr>
<td>Respiratory Tract</td>
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</table>

<table>
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<tbody>
<tr>
<td>Genito-Urinary Tract</td>
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</tr>
<tr>
<td>Intra-Abdominal</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory Tract</td>
<td>2</td>
</tr>
<tr>
<td>Skin and Soft Tissue</td>
<td>1</td>
</tr>
</tbody>
</table>

**Conclusion:** There were 30 infection episodes in 23 patients as described. Bacterial infections were more common in the immediate post-operative period, whereas viral infections were more common in the late post-operative period. There is limited data on infections post liver transplant in the indian scenario and this data can be used to plan appropriate preventive strategies.
A case of liver transplant in leptospirosis induced acute on chronic liver failure
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Apollo Hospital, Hyderabad, India

Background: Solid organ transplants are increasing in India. Guidelines on liver transplant postoperative infections are limited. We report a child with leptospirosis who underwent a liver transplant.

Methods & Materials: We review a case of a 12 year old male with Wilsons disease who presented with febrile illness and acute on chronic liver failure who was admitted at a tertiary care center and subsequently underwent a liver transplant.

Results: A 12 year old boy with Wilsons disease presented with a febrile illness and acute on chronic liver disease. He was evaluated for the cause of acute on chronic liver failure and found to have leptospirosis based on identifying spirochetes in the blood and urine. He was treated initially with cefaperazone-sulbactam and subsequently with doxycycline. His pretransplant period was complicated by Acinetobacter pneumonia and bacteremia. After optimizing the clinical condition he underwent a living donor liver transplant. He improved post-transplant for 2 weeks. Subsequently he developed CMV viremia, treated with ganciclovir. He developed multiple skin necrotic areas which was concerning for septic emboli. He had unexplained leukocytosis. Computerized tomography revealed cavitary lesions in the left upper lobe and right lower lobe, consolidation of left lower lobe, kidney infarction and mucosal thickening of the maxillary and sphenoid sinus. Due to the high risk of biopsy, bronchoscopy was done and bronchoalveolar fluid (BAL) was analyzed. Fungal stain of the BAL was positive and culture was suggestive of Mucor in both the left upper and lower lobes. He was started on Amphotericin B deoxycholate. Subsequently he developed seizures and had features of brain death, presumed due to spread of the mucormycosis.

Conclusion: There is no report of liver transplant post leptospirosis. This child underwent a liver transplant following leptospirosis in the background of Wilsons disease. He survived for a few weeks post-transplant but unfortunately died due to mucormycosis, a disease more common in a country like India. Here we were able to diagnose the mucormycosis ante-mortem with BAL and did not require tissue. Strong clinical suspicion, better diagnostics and early initiation of therapy is warranted. Unfortunately due to extensive fungal disease, we could not salvage him.
An unusual presentation of invasive aspergillosis - Diagnostic and management dilemmas

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Background: Invasive Aspergillosis is a fungal infection occurring with increased incidence in patients who are receiving chemotherapy, immunosuppressive therapy or long term corticosteroid therapy. Among these, it more commonly afflicts patients with pre-existing respiratory compromise such as COPD or bronchial asthma. Typically it presents with fever, cough, dyspnoea, pleuritic chest pain and occasionally with haemoptysis.

Methods & Materials: Case Report:
A 60 year old male patient presented to the emergency department with complaints of an intermittent, high grade fever for the past two days. A physical examination revealed only the presence of mild hepatomegaly. As part of a routine work up for pyrexia of unknown origin (PUO), a chest x-ray performed revealed multiple bilateral nodular lesions. A subsequent contrast enhanced CT scan of the chest showed bilateral heterogeneously enhancing mass lesions, with features suggestive of a neoplastic aetiology. The patient rapidly deteriorated, developing neurological symptoms, (GCS: 3). Brain imaging studies reiterated the high probability of metastatic lesions. However, in view of the patient’s symptomatology and his immunocompromised state, fungal assays were sent and serology revealed a positive Aspergillus antigen.

Results: We report an unusual presentation of Invasive Aspergillosis in a patient with well controlled Aplastic Anaemia. The initial diagnosis misleadingly pointed towards neoplastic lesions in the lung with metastasis to the brain.

Conclusion: This case exemplifies that a high index of clinical suspicion and an increased awareness of the possibility of invasive aspergillosis is paramount in improving patient outcomes. It also serves to show the
difficulties faced with the parallel use of different treatment regimens and how unconventional treatment protocols may sometimes prove effective.
Rare case of amphotericin-B resistant cryptococcal meningitis in HIV non reactive patient

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¹PGIMER & Dr. RML Hospital, New Delhi, Delhi, India, ²PGIMER & Dr. RML Hospital, Delhi, India

Background: Cryptococcal meningitis is caused by cryptococcal neoformans and cryptococcal gatti. The incidence of cryptococcus meningitis has risen dramatically in past two decades especially in Eastern Africa and South-East asia due to HIV epidemic and increasing use of immunosuppressive drugs. Infection usually acquired through inhalational route causing primary pulmonary cryptococcosis with dissemination to extra pulmonary sites in immunocompromised. Commonly presents with fever, headache, altered sensorium and neck stiffness. Very few cases of cryptococcal meningitis in Non-HIV patients that too with amphotericin resistance has been reported.

Methods & Materials: We are reporting one such case who presented with fever, headache, vomiting for three weeks without any history of exposure to bird excreta. Neck rigidity was absent. Fundus examination was normal, routine test was normal USG/ CXR (PA vie) with normal NCCT head.

Results: CSF routine microscopy showed 102mg/dl proteins and 74mg/dl sugar and CSF culture showed budding yeast cells with india ink preparation positive for cryptococcus. Cryptococcal antigen was positive in CSF but negative in serum. HIV (1&2) was negative (with ELISA and Western blot). CD4 count was 239/µL. TFT and S. IgG levels were normal. Patient was initiated on amphotericin-B and flucytosine and was continued for six weeks. India ink still came positive for cryptococcus. CSF Cultures were sent again which was positive for cryptococcus neoformans which was resistant to amphotericin-B but sensitive to flucytosine, fluconazole and voriconazole. CD4 counts were done again which were 629/µL. Her KFT was normal at all times and treatment was continued for further two weeks (total eight weeks). Patient improved clinically headache, fever, vomiting was relieved, did not give consent for repeat LP and so patient was discharged on request on fluconazole 400 mg/day. After two weeks patient came in altered sensorium with headache for one day. NCCT head was done which showed obstructive hydrocephalus and patient was posted for EVD but patient expired on second day due to brainstem herniation.

Conclusion: We hereby present this case to sensitive clinicians towards amphotericin resistant cryptococcal meningitis which is increasingly encountered in HIV as well as non-HIV patients.
Background: Chronic Hepatitis B infection (CHBV) is a leading cause of advanced liver diseases. Mother to child transmission accounts for most cases of CHBV infections. The risk is highest in HBsAg+ve and HBeAg+ve mothers (transmission rate: 70%–90%). Follicular T helper cells (TFH) have shown a preventive role against HBV through activating the B cell mediated humoral immunity in patients. Role of TFH cells in HBV transmission is elusive. Hypothesis: TFH cells are essential for preventing HBV transmission and impairment of these cells may lead to vertical HBV transmission.

Methods & Materials: HBsAg+pregnant mothers were followed up till delivery & babies were tested for evidence of HBV transmission (HBsAg or HBVDNA). Mothers were divided into 2 groups: Transmitting (N=21; Group A) & non transmitting (N=28; Group B). Phenotyping and functionality of TFH cells and its subsets along with co-stimulatory molecule ICOS was performed by flowcytometry. Signature cytokine of TFH cells IL-21 was measured by ELISA. NGS RNA sequencing was done in PBMCs in both groups. RNA sequencing & FACS data was validated by qRTPCR. Correlation of TFH with HBV DNA was done. Multivariate regression & ROC was drawn to determine cellular predictors of HBV transmission.

Results: CD4+CXCR5+(TFH) cells were significantly decreased in Group A compared to Group B(6.3± 4.4% vs.17.2±5%, p=0.001,FIG.1A) and ICOS expression on TFH was also decreased in Group A though it was not significant(Fig 1b). RNA sequencing data also showed >2 fold decreased expression of TFH cell related genes like CD4, BCL6, ICOS, SLAMF1, CXCR5 and BAFF, BATF, CD40, BAFFR in Group A than Group B(Fig 1c). qRT-PCR was done for validation of these genes (Fig 1d). IL-21 was also decreased in Group A(Fig1e). Reciprocal relation was found between TFH cells and HBV DNA(Fig 1f) and ROC showed significance of TFH cells with cutoff 9.5 values for prediction of HBV transmission with highest specificity & sensitivity.

Conclusion: Mother’s immunity plays an essential role in prevention of vertical HBV transmission. TFH cells can be used as a marker to predict HBV Transmission. Decreased and dysfunctional TFH cells in HBV infected mothers leads to greater chances of HBV transmission to their babies.
Significance of preservation fluid cultures in solid organ transplantation

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¹University of Illinois at Chicago, Chicago, Illinois, USA, ²University of Illinois Medical Center at Chicago, Chicago, USA, ³Central Michigan University, Saginaw, USA

Background: The study aim was to determine microbiological characteristics of preservation fluid (PF) cultures in solid organ transplant (SOT) patients and to evaluate their impact on mortality and morbidity.

Methods & Materials: A retrospective analysis of PF culture data for SOT recipients was performed between November 1, 2013 and November 1, 2014. Deceased donor culture data and PF was collected from the UNOS database. Additional PF culture data was obtained locally immediately upon organ arrival to the operating room.

Results: Analysis of data from 69 SOT recipients (Kidney 69.5%, Kidney-Pancreas 11.5%, Pancreas 7%, and Liver 12%) revealed 19 (27.5%) positive deceased donor cultures and 43 (62.3%) PF cultures. Microbial agents isolated from PF were coagulase-negative staphylococci (52.3%) Diphtheroids (39.5%), Staphylococcus spp., Pseudomonas spp., and yeast (all 9.3%). Three patients (4.3%) had same microorganism in deceased donor and PF culture. Of the 49 patients with positive cultures (either PF or deceased donor cultures), 21 (42.8%) received pre-emptive antibiotic therapy within 48 hours.

One renal transplant patient developed invasive Pseudomonas infection in the urine related to the deceased donor cultures. There were no other incidences of invasive infections related to the microorganisms isolated in deceased donor culture or PF culture regardless of administration of pre-emptive antibiotic therapy. Mortality rates at both 30 days and 90 days were not significantly different in recipients with positive or sterile PF cultures (30 days: 1 [2.3%] vs. 0 [0%], p=1; 90 days: 2 [4.6%] vs. 0 [0%], p=0.52). Rates of transplant graft loss at 30 days were also not significantly related to the rates of positive or sterile PF cultures (6 [13.9%] vs. 0 [0%], p=0.07).

Conclusion: Microbial contamination during solid organ transplantation occurs frequently. Although rates of mortality and morbidity remain low, virulent organisms such as Pseudomonas spp. can cause serious infections. Fatal consequences due to invasive infection can be avoided with appropriate and timely surveillance of microorganisms isolated from cultures of samples obtained from donors, grafts, and preservation fluid.
Plasmodium falciparum GLURP 2 clones on the Jos Plateau, Nigeria, possibly indicates that the region is a low endemic area.

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¹University of Calabar, Calabar, Nigeria, ²Jos University Teaching Hospital, Jos-Plateau, Nigeria

Background: Malaria is holoendemic in Nigeria. But since its transmission is also determined by geographical factors, the disease differs in its characteristics in different parts of the country. Genotyping the parasite is useful in studying its transmission dynamics. In high endemic areas, infections are often composed of multiple distinct parasite clones; while in low endemic areas the numbers of clones are usually few. On the Jos plateau, little is known about the genetic characteristics of Plasmodium falciparum. We thus set out to genotype Plasmodium falciparum isolates in patients with malaria, using the Glutamate Rich Protein 2 (GLURP 2) genes.

Methods & Materials: This was a hospital based, cross sectional study. Capillary blood samples were collected for microscopy and nested Polymerase chain reaction (PCR) from 200 eligible inpatients and outpatients at the Jos University Teaching Hospital (JUTH), Nigeria. Subjects were selected based on a history of fever or axillary temperature of more than 37.5°C at presentation, who were not on anti-malarial treatment or who had not received anti-malarial therapy in the two weeks preceding the study. Microscopy of giemsa stained films was carried out to provide early diagnosis. Samples for PCR were collected on filter paper and the methanol-heat extraction method was used to extract DNA from the dried blood spots. PCR was carried out using primers that are specific for region II of the GLURP gene under conditions recommended by the World Health Organization.

Results: Thirty (15%) out of the 200 subjects were positive for Plasmodium falciparum malaria by microscopy, while 34 (17%) out of the 200 were positive by PCR. Ten (29.4%) of the 34 samples positive by PCR showed infection by a single clone of Plasmodium falciparum; while the remaining 24 (70.6%) showed infection by two clones of Plasmodium falciparum.

Conclusion: The few numbers of clones of Plasmodium falciparum seen in this study suggests that the Jos plateau is a low malaria endemic area. Application of malaria control strategies as recommended for low endemic areas could be more effective in controlling malaria in this region.
Secondary syphilis manifesting as annular lichenoid plaques
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1Lokmanya Tilak Municipal General Hospital and Lokmanya Tilak Municipal Medical College, Sion Mumbai, Mumbai, Maharashtra, India, 2 Lokmanya Tilak Municipal General Hospital and Lokmanya Tilak Municipal Medical College, Sion Mumbai, Mumbai, India, 3 Lokmanya Tilak Municipal General Hospital and Lokmanya Tilak Municipal Medical College, Sion Mumbai, Mumbai, India

Background: Syphilis continues to be a diagnostic challenge as the myriad manifestations of secondary syphilis can mimic a lot many dermatological disorders. Annular Lichenoid syphilis is an uncommon entity, reported only occasionally in the penicillin era. We present the case of a 24-year-old man presenting with annular lichenoid lesions over face

Methods & Materials: A 24-year-old male with multiple pink to brown coloured lesions over face since 1 month. At the same time he was being treated for acne vulgaris with tretinoin 0.025% cream and clindamycin 0.05% cream from private practitioner. Lesion initially appeared as reddish coloured gradually spreading peripherally to become annular with central hyperpigmentation. Past history revealed homosexual unprotected sexual exposure with multiple partners, last passive exposure 1 month prior to onset of skin lesions. General and systemic examination was normal. On cutaneous examination there was evidence of multiple annular plaque over face with central hyperpigmentation. Also hyperpigmented macules over bilateral palms and soles, neck, chest, forearms, hands was present. Deep dermal tenderness was positive. Differential Diagnosis kept was Lichen Planus, Secondary Syphilis, Polymorphic light eruption disorder and Erythema Multiforme. Serological investigation showed Venereal disease research laboratory assay came out to be positive in the titres for the patient: 1:32, and Treponema pallidum haemagglutination for syphilis was Positive. Serology for Hepatitis B, and HIV was negative.

Results: Biopsy from annular plaque showed interface dermatitis, perivascular and perieccrine plasma cell infiltrate and granuloma. Diagnosis kept was Secondary syphilis presenting as unusual annular lichenoid lesions was favoured. Three doses of Intramuscular Injection of Benzathine penicillin 2.4 million units was given. After 3 months all skin lesions subsided with persistence of post inflammatory hyperpigmentation over face. 

Conclusion: The presentations of syphilis, often described as "the great imitator," can be varied and atypical. Thus prompt diagnosis and treatment is necessary.
Is there a correlation between Chlamydia trachomatis detection and development of disease in reactive arthritis/undifferentiated spondyloarthropathy patients

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¹National Institute of Pathology (ICMR), New Delhi, India, ²Army Hospital (Research & Referral), New Delhi, India

Background: The search for Chlamydia trachomatis or its components at the site of the primary infection or in the joint is the optimal approach to confirm chlamydial aetiology of Reactive Arthritis (ReA). The most specific diagnosis of C. trachomatis induced-ReA is made by detection of the pathogen in the joint itself. Undifferentiated Spondyloarthropathy (uSpA) patients generally have asymptomatic ReA. Also, the majority of infections are asymptomatic in both males and females, hence, diagnosis during these phase is a challenging task. 1 - 15% patients with genital infection subsequently develop acute arthritis which later becomes chronic. No diagnostic modalities are available till date for such cases. We aim to detect C. trachomatis using various diagnostic methods in Synovial Fluid (SF) and urine and correlate these findings with age, sex, duration/development of disease.

Methods & Materials: With the permission of hospital ethics committee, study was conducted in 115 arthritic patients, viz.: ReA/ uSpA (n- 45) / Rheumatoid Arthritis (n- 35) / Osteoarthritis (n- 35). SF/ urine samples were investigated for C. trachomatis infection by various molecular/ non-molecular methods of diagnosis, viz: nucleic acid amplification test, anti-C. trachomatis IgA antibodies and direct fluorescence assay. Data was clinically correlated and statistically analyzed.

Results: 91% (10/11) PCR-positive ReA/ uSpA patients for C. trachomatis in SF and 77% (10/13) anti-C. trachomatis IgA-positive patients had chronic infection (disease duration > 06 months) while 83.3% (5/6) patients positive for C. trachomatis by DFA in urine had an acute infection (disease duration < 06 months). Further, DFA in urine followed by PCR in SF was able to detect C. trachomatis infection in 83.3% (5/6), 63.6 (7/11), respectively in 18-30 years age group. 91% male ReA/ uSpA patients had C. trachomatis infection. 69.2% (9/13) ReA/uSpA symptomatic patients had anti-C. trachomatis IgA antibodies in SF.

Conclusion: This study demonstrated that PCR and anti-C. trachomatis IgA in SF might be useful techniques to differentiate between acute and chronic chlamydial infection, respectively; while male patients in the younger age group are more prone to develop C. trachomatis-induced ReA.
The evaluation and risk assessment of sexually transmitted disease in Korean adolescents at risk
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1Hallym university College of medicine, Seoul, Korea, Republic of, 2Ewha University, Seoul, Korea, Republic of

**Background:** There have been little researches of sexually transmitted infection (STI) for adolescent at risk in Korea and these studies were just for prevalence limited to Chlamydia trichomonas and Neisseria gonorrhoeae. We explored prevalences and risk factors of 10 pathogens of STI at risk adolescent. Through conducting treatment model, we aim to develop a substantial management plan.

**Methods & Materials:** Total of 237 subjects in one youth protection center and five probation offices were examined in Korea. First-voided urine specimens were collected for analysis of C. trachomatis, N. gonorrhoeae, Trichomonas vaginalis, Mycoplasma. hominis, Mycoplasma genitalium, Ureaplasma. urealyticum, Ureaplasma parvum by nucleic acid amplification tests using multiplex real-time PCR and VDRL titer, HSV-1 & 2 IgG and HIV Ag/Ab were tested by serum. Through anonymous, self-administered, structured questionnaire, basic characteristics and risk factors were collected. We conducted treatment model for adolescents who were infected with STI and agreed to treatment.

**Results:** The prevalence of C. trachomatis (13.9%) was the highest followed by N. gonorrhoeae (1.7%), T. vaginalis (0.8%), syphilis (0.8%), HSV (0.4%) and HIV (0%). The prevalence of U. urealyticum (24.7) was the highest in other infectious diseases followed by U. parvum (24.1%), M. hominis (17.3%), M. genitalium (4.2%). There were no significant association in family structure, economic status and psychologic factors with STI. Only past STI history was significant difference according to infection (OR = 7.039, 95% CI = 2.051-24.157, P <0.001). Total of 88 (66.2%) adolescents treated in our treatment model and 89.6% were satisfied with discovery of STI, connection to treatment and explanation of medical team.

**Conclusion:** Prevalence of STI at risk adolescents is similar level as adults in Korea and institutionalization of screening and treatment for adolescents is needed. Screening system should include at least chlamydia and gonorrhea and one stop system from screening to treatment will be effective.
Screening for chlamydia trachomatis in reproductive age group women by real time PCR assay in a semi-urban area of South India

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Background: Genital Chlamydia trachomatis presents as asymptomatic infection and hence if left untreated, leads to Pelvic Inflammatory Disease and Infertility. Centre for Disease Control and Prevention(CDC) has recommended that screening of women for Chlamydia trachomatis under the age of 24 years at least once in a year, as mandatory. In a developing country like India, due to inadequate data and resources, it is not currently practiced as a mandatory screening procedure. Hence this study is aimed at estimating a reliable laboratory based data on Chlamydia trachomatis infection in reproductive age group women (18 to 45 years) attending a tertiary care hospital in a semi urban area of South India.

Methods & Materials: A cross sectional study was conducted on 110 women attending gynecology OPD who has fulfilled the inclusion criteria over a period of one year from April 2014 to March 2015. After obtaining informed consent, under aseptic precautions, samples were collected from endocervix with the help of cytobrush and inoculated in a sterile aliquot tube containing 2ml of 99% ethanol for the detection of Chlamydial nucleic acid by Real time PCR.

Results: 8.18% (9/110) showed positivity for Chlamydia trachomatis by Real time amplification plot analysis. All the infected population belonged to reproductive age group less than 30 years.

Thermal profile of run of Real time PCR for Chlamydia trachomatis

Amplification plot of Real time PCR assay for Chlamydia trachomatis

Conclusion: Previous studies have proved that available diagnostic techniques like ELISA are not reliable indicators and nowadays molecular methods are the choice for an appropriate diagnosis. The proportion of Chlamydia trachomatis obtained in our study emphasize that health programmes should be implemented to screen the clinically silent Chlamydia trachomatis infection in women of reproductive age group less than 30 years to safeguard the reproductive health of women.
Differential expression of superoxide dismutases in early aborters infected with Chlamydia trachomatis

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Background: Chlamydia trachomatis (Ct) can infect placenta/decidua causing Spontaneous Abortion (SA). However, associated pathogenic mechanisms are unknown. Placental oxidative stress has been implicated in pathophysiology of abortion. It was hypothesized that oxidative stress-induced placental dysfunction may be cause of multifactorial/polygenic etiologies of abortion. Study aimed to evaluate role of Superoxide Dismutases (SODs) in pathophysiology of early abortion by studying expression of Manganese-Superoxide Dismutase (Mn-SOD) and Copper, Zinc-Superoxide Dismutase (Cu, Zn-SOD) in Ct-infected women.

Methods & Materials: With hospital ethics permission, Endometrial Curettage Tissue (ECT) was collected from 145 aborters (Sporadic Spontaneous Aborters, SSA and Recurrent Spontaneous Aborters, RSA) presenting with vaginal bleeding/undergoing incomplete SA in first trimester of pregnancy and 120 aborters undergoing MTP at Department of Obstetrics and Gynaecology, Safdarjung hospital, New Delhi (India). Group I comprised of Ct-infected SSA/ RSA while uninfected induced aborters were included in Group II. PCR assay was performed for diagnosis of Ct cryptic plasmid (200 bp). Qualitative/quantitative expression of Mn-SOD/ Cu, Zn-SOD mRNA was assessed by Reverse Transcription (RT)-PCR/ Taqman real-time PCR (q-PCR).

Results: Infection with Ct was found in Group I (13.7%; n= 20/145) patients, viz.: SSA and RSA (11/85 and 9/60, respectively), whereas none of controls were infected (Group II). Qualitatively, both Mn-SOD and Cu, Zn-SOD (p < 0.001) showed upregulated expression in Group I (Ct-infected SSA/ RSA) versus Group II (control) patients; however, level of Mn-SOD was significantly increased (p < 0.0001) in comparison to Cu, Zn-SOD. Similar findings were obtained by qPCR analysis as there was significant differential expression of transcript level of SODs, viz.: Mn-SOD (p < 0.0001) and Cu, Zn-SOD (p < 0.05) in infected SSA/ RSA patients (Group I). Ct-infected RSA showed elevated Mn-SOD expression (p < 0.001) as compared to infected SSA. However, no significant difference was found in expression of Cu, Zn-SOD between infected patients, viz.: RSA versus SSA.

Conclusion: The study suggested that superoxide radical and its scavenging system play important role in maintenance of pregnancy in Ct-infected women. Overall results indicated that both SODs are differentially expressed and Mn-SOD plays greater protective role in Ct-associated early abortion.
Hepatitis B and Human immunodeficiency virus co-infection among pregnant women in resource limited high endemic setting, Addis Ababa, Ethiopia: Implications for current and emerging prevention and control measures
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Background: Pregnant women infected with hepatitis B virus (HBV) and HIV can transmit the infection to their fetuses and newborns. The study was intended to determine co-infection of hepatitis B and Human immunodeficiency virus and assessing risk factors among ANC visiting women.

Methods & Materials: A hospital based cross-sectional study was employed from July to October, 2014 in a total of 215 pregnant women. Enzyme Linked Immunosorbent Assay kit was used to detect HBV seromarker and HIV antibody in serum. Descriptive and multivariate logistic regression was performed and P<0.05 considered as indicator of statistical significance.

Results: The prevalence of hepatitis B infection in the study area was 6%. High HBV positivity was recorded among the age group 25 –29 years. Many risk factors for HBV acquisition exists in study settings, but amongst these, history of abortion (AOR=19; CI=2.78-130.367; P-value=0.003), surgery (AOR=9.8; CI=1.392-69.610); P-value=0.0.022) and tattooing (AOR=7.7; CI=1.185-50.28; P-value=0.033 were significantly associated with HBV infection. The prevalence of HIV infection was 4.19 % and none of the socio-demographic and the risk factors were associated with HIV positivity.

Conclusion: The prevalence of hepatitis B among in the study area was intermediate and the prevalence of HIV/HBV co-infection was 2 (22.2%). This may warrant patterns toward high infectivity and therefore maximized risk of perinatal infection transmission. These capitalize the demand to step up preventive efforts against both infection and perinatal transmission in our study set up and the country.
Background: Yellow fever vaccine (YFV) had been considered the safest of the live virus vaccines. But, Yellow fever vaccine - associated neurotropic disease (YEL - AND), previously called as post - vaccinial encephalitis is a known rare adverse reaction to this vaccine.

Methods & Materials: A 30 years old hypertensive male presented to us with high grade intermittent fever, severe headache & multiple episodes of vomiting three days after receipt of a 17D strain live attenuated yellow fever vaccine at a government hospital. On examination he had neck stiffness with positive Kernig’s sign without any focal neurological deficit. He was previously diagnosed as having chronic kidney disease 3 mnths ago & was on medical follow up.

Results: Blood investigations showed a normal hemogram, liver function tests and a borderline raised creatinine (1.8 mg/dL). Blood investigations sent for the endemic tropical diseases like malaria, dengue & leptospirosis came out negative. Cerebrospinal fluid (CSF) was suggestive of viral meningitis (protein 101 mg/dL, sugar 50 mg/dL, total cells 82) with lymphocyte predominance. His CSF culture showed no growth. MRI Brain was normal. He was treated symptomatically. His symptoms resolved over next 2-3 days & he was discharged without any focal neurological deficit. We believe that he suffered a rare complication of yellow fever vaccine - YEL- AND since no other cause was found.

Conclusion: The two important complications of YFV are YEL-AND & Yellow fever vaccine associated viscerotropic disease (YEL – AVD). The incidence of YEL - AND in United States is 0.8 per 1,00,000 doses administered. It represents a conglomerate of clinical syndromes, including meningoencephalitis, Guillain-Barré syndrome, acute disseminated encephalomyelitis, and rarely, cranial nerve palsies. The onset of illness for documented cases is 3–28 days after vaccination. It is rarely fatal & is treated symptomatically.
Mathematical modelling of the effects of prebiotic concentration on lactobacillus casei growth

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**Background:** Probiotics are microorganisms that are beneficial to the human digestive system. *Lactobacillus Casei* is a beneficial probiotic bacterium present naturally in the human digestive system. Also, *Lactobacillus Casei* helps in treatment of several gastric disorders and in inhibiting the growth of *Helicobacter Pyroli* that causes gastro-intestinal ulcers. Prebiotics, in general, refers to substances that help in stimulating the growth of the beneficial probiotic bacteria. Further, the growth of probiotics is sustained by the optimized concentration of prebiotics.

**Methods & Materials:** In this work, the growth of *Lactobacillus Casei*, in response to the prebiotic concentration is obtained experimentally. The growth curves of *Lactobacillus Casei*, in response to a 10% nutrient solution (10grams of milk powder in 100ml) was obtained using UV spectrophotometer (Model: UV 1800) under sterile laboratory conditions. The absorbance was measured at a wavelength of 600nm for a time period of 150 minutes. Further, using the measured data, the growth of *Lactobacillus Casei* in response to the nutrients was modeled using a mathematical modeling approach namely the transfer function model.

**Results:** Results demonstrate that the developed model is efficient in capturing the dynamics of *Lactobacillus Casei* growth, with an accuracy of 88.81%. Further, by analyzing the model, it was found that the growth of *Lactobacillus Casei* is stable. Hence, the model can be efficiently used to develop suitable control systems for sustaining the growth of *Lactobacillus Casei*.

**Conclusion:** This work appears to be of high clinical and industrial importance since *Lactobacillus Casei* helps in treatment of several gastric disorders and the development of mathematical models of probiotic growth is highly useful for mass production of probiotics.
Low and declining attack rates of imported typhoid fever in the Netherlands despite restrictive vaccination policy
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Background: Typhoid fever occurs mainly in the developing world where sanitary conditions remain poor. In Western countries typhoid fever occurs mainly in returning travelers or their direct contacts. The aim of this study was to evaluate the current Dutch guidelines for typhoid vaccination.

Methods & Materials: Crude annual attack rates (AR) per 100,000 Dutch travelers were calculated during the period 1997 to 2014 by dividing the number of typhoid fever cases by the estimated total number of travelers to a specific country or region. Regions of exposure and possible risk factors were evaluated.

Results: During the study period 607 cases of typhoid fever were reported. Most cases were imported from Asia (60%). Countries with the highest AR’s were India (30), Indonesia (11) and Morocco (11). The absolute number of typhoid cases reported in the Netherlands declined significantly ($p < 0.001$), despite an increase in the number of travelers. There was a significant decline in AR’s among travelers to Morocco, Turkey and Indonesia, which are popular travel destinations. AR’s among travelers to intermediate risk areas according to the LCR guidelines (Figure 1) like Latin-America or Sub-Saharan Africa were very low (0.3 and 2 respectively), despite the restrictive vaccination policy to these areas. Almost half of the cases were ethnically related to typhoid risk regions and 37% were cases visiting friends and relatives.

Conclusion: The overall AR of typhoid fever in returning travelers to the Netherlands is very low despite the restrictive vaccination policy compared to some other countries. There has been a significant decrease in overall number of cases. The Dutch vaccination policy not to vaccinate short-term travelers to Latin-America, Sub-Saharan Africa, Thailand and Malaysia seems to be justified, because the AR’s for these destinations remain very low. These results might suggest that further restriction of the Dutch vaccination policy is justified. Furthermore, the available data helps to specify risk regions for Dutch travelers.
Novel synthetic antimicrobial peptides against streptococcus pneumoniae
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**Background:** According to WHO, 1.6 million deaths are caused by pneumococcal infections every year with 0.7 to 1 million in children younger than 5 years mostly in Asia and Africa. Like other gram positive bacteria, *Streptococcus pneumoniae* is increasingly difficult to treat due to the irrational use of antibiotics. Antimicrobial peptides (AMPs) represent a possible alternative for current antibiotics against drug resistant pathogens.

**Methods & Materials:** In this study, thirteen antimicrobial peptides were designed based on two natural peptides indolicidin and ranalexin. The in vitro activity of these peptides was investigated using broth microdilution assay, hemolytic activity assay, time killing assay, and toxicity assay against two cell lines WRL-68 and NL-20. Mechanisms of action of peptides were assessed using transmission electron microscopy (TEM), scanning electron microscopy (SEM), DNA binding assay, and in silico molecular docking against three virulent factors.

**Results:** Our results revealed that four hybrid peptides RN7-IN10, RN7-IN9, RN7-IN8, and RN7-IN6 possess potent antibacterial activity against 30 pneumococcal clinical isolates (MIC 7.81-15.62 µg/ml). These four hybrid peptides showed broad spectrum antibacterial activity (7.81 µg/ml) against *S. aureus*, methicillin resistant *S. aureus* (MRSA), and *E. coli*. Furthermore, the time killing assay results indicated that the hybrid peptides were able to eliminate *S. pneumoniae* within less than one hour which is faster than the standard drugs erythromycin and ceftriaxone. The cytotoxicity was tested against human erythrocytes, WRL-68 normal liver cell line, and NL-20 normal lung cell line. The results revealed that none of the thirteen peptides have cytotoxic or hemolytic activities at their MICs.

TEM and SEM results showed that these four peptides are killing the bacteria by destroying the integrity of their membranes. DNA binding assay revealed that the hybrid peptides were able to bind to DNA at 62.5 µg/ml preventing it from migration through the agarose gel.

![Image 1](image1)

![Image 2](image2)

![Image 3](image3)

The effect of hybrid peptides on the integrity of bacterial membranes
Conclusion: In conclusion, our results indicated that hybrid peptides RN7-IN10, RN7-IN9, RN7-IN8, RN7-IN7 and RN7-IN6 represent promising first templates for developing a new class of antibacterial agents against *S. pneumonia*. Hence, with the growing resistance to traditional antibiotics, our peptides can offer an alternative to today’s antibiotics to protect against resistant bacteria.
Investigation of diverse carbapenem resistance mechanisms in pseudomonas aeruginosa isolated from a tertiary care centre in India

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Background: Emergence and spread of carbapenem resistance in Psuedomonas aeruginosa is on the rise. Resistance mechanisms for carbapenems are of various types. The most common are carbapenemase production, loss of porins and over-expression of efflux pumps. Presence of each of these resistance mechanisms contributes to various phenotypes such as imipenem resistant but meropenem susceptible (IRMS), meropenem resistant but imipenem susceptible (MRIS) and resistant to both imipenem and meropenem (IRMR). This study was undertaken to investigate the resistance mechanisms responsible for these different phenotypes.

Methods & Materials: A total of 50 carbapenem resistant isolates of P. aeruginosa were collected from blood and respiratory samples of patients with ventilator associated pneumonia and/or bacteremia. Of the fifty isolates, 8 were IRMS, 3 were MRIS and 39 were IRMR phenotypes. Carbapenemase genes such as blaSPM, blaIMP, blaVIM, blaNDM, blaKPC and blaOXA-48 were screened for all the 50 isolates. In addition, for 8 IRMS, sequencing of OprD was done to look for the presence of mutations and relative quantification was done to quantify the OprD transcripts. While for three MRIS, relative quantification of mexAB efflux pump was done to quantify the over expression.

Results: Of the 50 isolates, carbapenem resistance due to carbapenemase enzymes was found to be only 22%, which was due to blaVIM (n=7), blaNDM (n=3) and blaOXA-48 (n=1). Among the 8 IRMS isolates, only one was a blaVIM producer and seven isolates harbored multiple mutations in oprD gene. In addition, quantification revealed that, down regulation of oprD gene transcripts were found in few isolates. While for MRIS isolates, mexAB pump was found to be over-expressed.

Conclusion: From this study we found that, IRMS is primarily due to the mutations across various regions in the loops of OprD gene, MRIS is due to the over expression of mexAB efflux pumps and IRMR is due to carbapenemases. This study results confirms that, these phenotypes are due to the different resistance mechanisms. This study also emphasizes that preliminary disc diffusion should be performed for both imipenem and meropenem particularly for P. aeruginosa, rather than testing single agents, as the resistance mechanisms for each of the agents are different.
Demonstration of horizontal gene transfer of fluoroquinolone resistance by plasmids in clinical isolates of shigella spp. and salmonella spp.

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Background: Shigella and Salmonella infections represent a major health problem, particularly in the developing countries. 165 million cases of Shigella infection occur annually worldwide with 1.1 million deaths with most of the causality in children under 5 years of age. Plasmid mediated fluoroquinolone resistance is on the rise among enteric pathogens. This study was aimed to investigate the horizontal transfer of fluoroquinolone resistant gene harbouring plasmid from multi-drug resistant (MDR) Shigella spp. to susceptible Salmonella spp., which are closely related among diarrheal infections.

Methods & Materials: A total of 67 MDR Shigella spp. were isolated from faeces samples from January to December 2014. Isolates were identified by standard biochemical tests and further serotyped by slide agglutination test with commercial antisera. Antimicrobial susceptibility testing was performed as per Kirby-Bauer disc diffusion method. 20 MDR Shigella spp were selected in random for further analysis. Quinolone resistance genes (qnrA, qnrB and qnrS) were identified by colony PCR for all isolates. Plasmids were isolated from 20 isolates for confirmation of the presence of qnr genes. MDR Shigella isolates (n = 2), harbouring plasmid with qnrS gene was conjugated with completely susceptible Salmonella spp. to observe horizontal gene transfer. Selection of conjugates were done using antibiotic medium. Next generation sequencing (NGS) was performed to confirm the identity of the conjugated plasmid.

Results: 6 out of 20 MDR Shigella isolates had qnrS genes in addition to one qnrB gene in one isolate. The qnrS containing plasmid was observed to successfully transfer from MDR Shigella isolates (n = 2) to susceptible Salmonella spp. through conjugation. PCR of the transferred plasmid confirmed the presence of qnrS gene. NGS confirmed that the same qnrS harbouring plasmid was transferred from Shigella spp. to Salmonella spp.

Conclusion: This study clearly demonstrates that the plasmid donated by Shigella spp. can be naturally acquired by Salmonella spp., which poses a greater threat for rapid spread of fluoroquinolone resistance among enteric pathogens. Continuous surveillance of plasmids containing antimicrobial resistance genes is crucial for control of further spread of fluoroquinolone resistance.
Molecular binding analysis of aminoglycoside N-acetyltransferase aac(6')-Ib and its bi-functional fluoroquinolone active variant aac(6')-Ib-cr active-site with ciprofloxacin and kanamycin: An in-silico approach
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Background: Fluoroquinolone resistance is a serious public health problem. Enzymatic modification of fluoroquinolones is a new phenomenon discovered recently and is mediated by bi-functional fluoroquinolone active cr variant of aac(6')-Ib encoded fluoroquinolone modifying enzyme. The kinetic characterization of aac(6')-Ib-cr and its wild-type parent enzyme has been reported previously however its mode of action is still unclear. Hence, this study was carried out to understand fluoroquinolone acetylation mechanism using the predicted structure of aac(6')-Ib-cr via relevant in-silico methods.

Methods & Materials: The structure of aac(6')-Ib enzyme, retrieved from PDB database and predicted structure of aac(6')-Ib-cr (Yugendran T, Unpublished data) were docked against kanamycin and ciprofloxacin using Schrödinger. The complexes were analysed for protein – ligand interactions using Discovery Studio v3.5.

Results: The predicted structure of the mutant enzyme was docked with ciprofloxacin and its interactions were compared with that of kanamycin bound WT enzyme [PDB ID: 1VOC] to understand the mechanism underneath the development of resistance to ciprofloxacin. They were further cross referenced with interactions observed in ciprofloxacin docked WT enzyme. Results based on GLIDE score, complex energy and non-bonded interactions show that ciprofloxacin binds effectively with mutant enzyme and it is positioned close to acetyl-CoA so as to enable acetylation of fluoroquinolone, thereby rendering resistance to the drug. The key residues involved in interaction with ciprofloxacin were W75, G76, Y91, R128 and D141. Comparison with kanamycin bound WT shows that ciprofloxacin engages in more number of strong non-covalent bonds that could be the structural reason for enhancing strong affinity for ciprofloxacin towards mutant enzyme.

Conclusion: The role of T128R mutation in promoting the interaction with fluoroquinolone molecule has been revealed. The amino-acid residues that bring about the acetylation of fluoroquinolone by interacting with them have also been identified. The mechanism elucidated here ascertains the adaptive potential of aminoglycoside N-acetyltransferase that in this case is presumably driven by the selective pressure of fluoroquinolone use.
Combination of NS1 antigen ans anti-NS1 IgA assays in the diagnosis of dengue infection in Asia

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**Background:** Diagnosis of Dengue infection is essential for clinical care of patients with acute febrile illness. It has been demonstrated that combining NS1 Ag to an early serologic test improved Dengue diagnosis. If IgM has been extensively evaluated, there are few data about IgA detection in association to NS1. The purpose of these clinical studies was to evaluate the efficiency of combining dengue NS1 Ag and anti-NS1 IgA antibodies for the detection of Dengue infection during the acute phase in Asian populations.

**Methods & Materials:** Evaluated kits were Platelia™ Dengue IgA Capture associated to Platelia™ Dengue NS1 Ag for ELISA part and RDT Dengue IgA/IgG associated to Dengue NS1 STRIP for rapid testing. Sensitivity was performed on 216 samples from patients with clinically confirmed Dengue of 3 Asian countries: Cambodia (n=135), India (n= 31) and Singapore (n=50). Specificity was evaluated on 101 sera from 50 healthy Indian donors and 51 febrile patients from Cambodia for which dengue infection has been excluded.

**Results:** Depending on the population, the sensitivity ranged from 48.5 % to 93.3 % for Platelia™ Dengue IgA Capture assay versus 48.9% to 90.3% for IgM ELISA and 50 % to 100 % for the IgA rapid test. When NS1 and IgA tests were combined, the sensitivity reached 93.5% to 98.0% for the ELISAs and 94.1% to 100% for the rapid tests, compared to 94.9% for an “IgM+NS1” combination. The overall specificity of the NS1 and IgA combination in ELISA and rapid test assays were 95.9 % and 96.0 % respectively. The analysis of NS1 and IgA ELISAs sensitivity related to the sampling time after fever onset in Cambodia population showed that NS1 assay sensitivity was lower and decreased earlier (day 5) in secondary infection than in primary one (day 11).

**Conclusion:** Detection of anti-NS1 IgA efficiently completes NS1 antigen detection in the diagnosis of acute dengue infection in Asian populations. The combination performs as well on ELISA as on rapid test format and demonstrates similar performance to “IgM+NS1”. Moreover, IgA appears to be especially useful in secondary infections and has also been described to be indicative of more severe outcome in primary infections.
Atypical presentation and nosocomial spread - intensifying the MERS mystery and misery
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Background: Infection control measures to prevent nosocomial transmission of novel pathogens like the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) require strict adherence to guidelines. However, atypical presentations may mislead unwary Emergency Department (ED) physicians, thus posing challenges. We present the investigation of a MERS case with atypical presentation at the King Fahad Medical City (KFMC) in Riyadh in the summer of 2015.

Methods & Materials: The patient's charts and electronic health records covering her two ED visits and subsequent intensive care unit (ICU) admission were reviewed. Adhering to MOH protocols, health care workers (HCWs) exposed to the patient were monitored for possible nosocomial MERS CoV transmission.

Results: The patient was a 77-year-old female with Diabetes Mellitus, Hypertension, chronic kidney disease and chronic myelocytic leukemia who presented twice at the ED, within 4 days. On her first visit, she was febrile (37.9°C), had abdominal pain and distension (ascites), nausea and vomiting. Four days earlier, she had visited her primary hospital, known to be experiencing a MERS outbreak at that time, for chemotherapy. Biochemical and microbiological testing of drained ascitic fluid were unremarkable. She was discharged the same day after spending 10 hours in the ED. Three days later, she returned to the ED with progressive abdominal distension, worsening fever (38.8°C) and deteriorating hepatic and renal function. She developed pulseless electrical activity (PEA) and asystole that required resuscitation for 19 minutes. She survived the arrest but clinically worsened and died 4 days in the ICU. Despite 6 intra-hospital transfers (5 prior to MERS CoV confirmation) during her second visit, none of the exposed HCWs (n = 60) developed MERS; included are those who performed high risk procedures (intubation and CPR) on her. However, epidemiological investigation suggests she infected a post-mastectomy patient that shared the waiting room with her while awaiting triage on her first ED visit. Both patients died.

Conclusion: This case of an atypical MERS case with multiple exposures to several HCWs having varying levels of protection on multiple occasions led to only one nosocomial case thus further intensifying the mystery surrounding MERS CoV transmission.
The impact of increased use of ASHAs on rural immunization coverage in India

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Background: Accredited Social Health Activists (ASHAs) are located in rural Indian villages to promote positive health behaviors and facilitate better health care utilization, including vaccination. We calculated the average coverage of the Bacillus Calmette-Guérin vaccine (BCG); Diphtheria, Pertussis, and Tetanus vaccine (DPT); polio vaccine; measles vaccine, and full vaccine coverage (BCG, 3 doses of DPT, 3 doses of polio, and measles vaccine) across districts in India, and evaluated the impact of expanded ASHA presence on changes in district-level vaccine coverage.

Methods & Materials: We used District Level Household and Facility Survey data, collected in 2007-2008 (DLHS-3) and 2012-2013 (DLHS-4); districts are the unit of analysis. The changes in use of ASHAs and in vaccine coverage over time were calculated as the difference between district-level values in DLHS-3 and DLHS-4. For the logistic regression analyzing the relationship between expanded ASHA coverage and increased vaccination coverage, we dichotomized the change in ASHA presence at the median, and split vaccine coverage into two groups: increased and decreased vaccine coverage.

Results: Across the 267 districts in 21 states studied, 40.83% of villages within districts had ASHA workers in DLHS-3 on average, compared to 77.83% in DLHS-4. From DLHS-3 to DLHS-4, the average district-level coverage changed from 93.04% to 88.73% for BCG, 77.81% to 78.46% for DPT, 78.35% to 80.21% for polio vaccine, 80.93% to 79.08% for measles vaccine, and 62.88% to 50.95% for full vaccination. Greater than median increases in ASHA presence (≥30%) within a district were associated with 1.733 greater odds of increase in DPT coverage (95% CI: 1.036, 2.900) and 2.042 greater odds of increase in measles vaccine coverage (95% CI: 1.215, 3.430).

Conclusion: Expanded ASHA coverage was not significantly associated with changes in BCG, polio vaccine, or full vaccination coverage. ASHAs are essential to connecting families in rural areas with health care information and services. A critical aspect of their position is the promotion of vaccines. Significant associations between increased presence of ASHA workers and increased DPT and measles vaccination coverage at the district level in India corroborate the importance of their work and may call for expansion in their numbers and their role.
Tracking the emergence of multidrug resistant, extraintestinal pathogenic Escherichia coli in India

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Background: It is important and rather need of the hour to unravel the risk of antimicrobial resistance from a public health perspective in high burden countries such as India. The clinical significance of isolating multi-drug resistant (MDR) E. coli from hospitalized patients merits sustained attention. The objective of this study was to describe the evolution of antibiotic resistance and to gather evidence on spread of antimicrobial resistance by clonal expansion of clinical E. coli lineages.

Methods & Materials: A collection of 426 E. coli isolates was analysed as three subsequent batches from two tertiary care hospitals in India. The isolates were characterized by ERIC-based fingerprinting, O typing, phylogenetic grouping, MLST, virulence profiling, phenotypic and genotypic antimicrobial resistance analysis and whole genome sequence (WGS) analysis. Our integrated data were representative of a larger study aimed at understanding transmission dynamics, population genetic structure and shaping of antimicrobial resistance mechanisms in extraintestinal pathogenic E. coli (ExPEC).

Results: We found that the resistance to unrelated antimicrobials is rapidly emerging in the form of ESBL-MDR phenotypes (58%). The molecular epidemiology of antimicrobial resistance has undergone a rapid change over the course of our study with increase in ESBL, MDR and MBL rates from 31%, 50% and 0% to 86%, 95% and 28% respectively, as observed among the isolates studied in 2009 and 2012. Also the faecal E. coli isolates (37%) exhibited the acquisition of new ESBL variants such as CTX-M-15 instead of the classical TEM and SHV types. Phylogenetic analysis revealed that groups B2 and A were prevalent among clinical E. coli isolates (49% and 27% respectively). REP-PCR, MLST and PFGE analyses suggest that ExPEC strains are undergoing a sub-clonal evolution as demonstrated by the identification of ST131 E. coli in our collection (42%). E. coli ST131 was initially identified to be linked to the spread of fluoroquinolone resistance and CTX-M-15 extended-spectrum β-lactamase (ESBL) production. Alarmingly, ST131 strains harbouring NDM-1 (1.5%) carbapenem resistance were also identified.

Conclusion: This study suggests that clinical E. coli from India are strongly associated with CTX-M-15 enzyme and are clonally evolving with a potential for newer acquisitions and wide spread dissemination of antimicrobial resistance genes.
Healthcare worker exposure to solid organ recipients with Rabies virus disease: An infection control perspective  
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**Background:** The Rabies virus is transmitted to humans via bites or scratches from infected animals. When disease develops it is considered incurable. The Rabies Virus Disease (RVD) is preventable through vaccination or immunoglobulin administration immediately after exposure. Even though, RVD transmitted via solid organ transplantation has been documented in literature, the actual exposure risk to Healthcare workers (HCWs) remains undetermined. This study reviews the intervention and outcome following an infection control investigation of two solid organ recipients who developed RVD from a single donor. Two months after relocating from India to a neighboring gulf country, the donor died after presenting with nonspecific symptoms that included encephalitis. The donor's organs were harvested, with the heart and liver sent to a large teaching hospital located in Riyadh, Saudi Arabia. It was not until the RVD investigation was undertaken that it was known that the donor had been bitten by a dog approximately three months prior to death.  

**Methods & Materials:** A retrospective chart review and interviews were undertaken to identify those exposed to recipients suspected RVD. RVD was confirmed by brain biopsy. Review of the Safety Reporting System (SRS) was undertaken to identify any HCWs exposed to the recipient’s blood or body fluids. A screening tool for HCWs was developed to identify the level of exposure risk, and if post-exposure prophylaxis (PEP) was required. A Rabies Clinic was implemented during the investigation period to facilitate the interview and screening process. Collaboration between departments was required to facilitate the investigation, screening, and PEP process.  

**Results:** 294 HCWs were identified as having physical contact with the organ recipients. 272 HCWs were considered low risk not requiring PEP. 12 were lost to follow-up due to resignation. 10 HCWs received PEP: 3 due to a high risk procedure, 2 with an unreported splash exposure, and 5 due to an unsure exposure risk. 5 HCWs indicated inappropriate personal protective equipment use. No HCW developed RVD.  

**Conclusion:** The investigation identified areas for improvement; poor compliance with infection control practices, under-reporting of exposures, and organ donations from high risk countries to include screening for RVD if cause of death is associated with non-specific encephalitis.
Real time antimicrobial resistance surveillance in critical care: Identifying outbreaks of carbapenem resistant gram negative bacteria from routinely collected data
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Background: Statistically significant variation in antimicrobial resistance (AMR) occurs between hospitals, within hospitals, and over time. Whilst case mix and antimicrobial use contribute, the impact of cross-transmission on these fluctuations is not well understood. We investigated the utility of applying a statistical algorithm to identify outbreaks of carbapenem-resistant infections across three critical care units in a multi-centre teaching hospital network serving a population of 2 million in London, UK.

Methods & Materials: We applied a negative binomial regression model which accounts for seasonality and linear trends, as described by Noufaily et al., to routinely collected microbiology data (fiscal years 2009-2015 for two units, 2012-2015 for the third) for carbapenem-resistant Pseudomonas spp. and Enterobacteriaceae (CRE). The first two years of data for each unit was used to train the algorithm. Exceedances (i.e. weeks with possible outbreaks) were validated by antibiogram comparison (as a proxy-indicator of strain similarity), against hospital infection control reports, and where available through genotypic typing.

Results: Across the three units, 154 CRE (from 3640 Enterobacteriaceae) were identified. The algorithm identified 17 exceedance weeks, in 11 multi-week clusters. In four of these clusters (three K. pneumoniae, one E. coli) organisms shared identical antibiograms; typing was available for one K. pneumoniae cluster, indicating clonal NDM cross-transmission, and this was the only outbreak (of the 11 clusters) identified in hospital infection control reports. Among 786 carbapenem-resistant Pseudomonas spp. (from 2378 isolated), 27 exceedance weeks were detected, in 15 multi-week clusters. Organisms in eight clusters shared identical antibiograms. No typing was available and none of the clusters had been identified in hospital infection control reports. No additional outbreaks of CRE or carbapenem-resistant Pseudomonas spp. were identified through routine surveillance or in hospital infection control reports.

Conclusion: The rise of carbapenem resistant organisms necessitates low-cost, easy-to-use surveillance mechanisms to aid early identification of outbreaks, particularly in critical care. Our data suggests such outbreaks may be more common than previously thought, and may be going undetected by current surveillance systems. Application of the Noufaily algorithm to routinely collected microbiology data provides a valid mechanism to better target limited hospital epidemiology, infection control, and diagnostics resources.
Relationships between flavivirus serological laboratory test results from dengue endemic areas of India: Limitations and challenges

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Background: Cross-sectional, population-based seroprevalence studies provide data on exposure to pathogens, susceptibility and disease transmission dynamics, and are useful in public health and vaccination planning. Cross-reactivity between flavivirus IgG antibody assays is an important consideration where multiple flaviviruses co-circulate.

Methods & Materials: An age-stratified dengue and Japanese encephalitis virus (JEV) IgG seroprevalence study was conducted in 8 sites across India, enrolling 2,591 subjects aged 5 – 10 years. Sera were tested using commercial ELISA kits; those dengue positive were subjected to plaque reduction neutralization test (PRNT) for serotype-specific neutralizing antibodies (DEN-1, 2, 3 and 4). A threshold of ≥ 10 (1/dil) was considered detectable; an algorithm was applied to interpret profiles as “naïve”, “monotypic” or “multitypic”. This secondary analysis explored a hypothesis that JEV IgG status was associated with cross-reactive dengue antibodies. JEV IgG results were analyzed by: a) dengue IgG status; b) naïve/monotypic/multitypic PRNT profile; c) the number of serotypes with detectable neutralizing antibodies; and d) geometric mean neutralizing antibody titer (GMT). Associations were tested by Pearson’s chi squared test.

Results: Overall, 1,525/2,558 (59.6%) of available samples were dengue IgG positive, and 345/2,544 (13.6%) were positive for JEV IgG. Of JEV positive samples, 327 (94.8%) were also dengue IgG positive. Similarly, 96.5% of the 405 “inconclusive” JEV samples were dengue positive. Of the 1,794 JEV IgG negative samples, 801 (44.6%) were dengue IgG positive (p<0.0001). Examining PRNT profiles, 0.62%, 25.3% and 74.1% of JEV positive samples were naïve, monotypic and multitypic, compared to 4.5%, 38.6% and 56.9% of JEV negative subjects (p<0.0001). 97.8% of the JEV positive and 92.5% of inconclusive samples had detectable titres against all four dengue serotypes, compared with 71.0% of the JEV negative samples. For every monotypic dengue serotype, the GMT was highest in JEV positive subjects, followed by those with an inconclusive JEV result, and lowest in the JEV negative group.

Conclusion: While limited by a lack of PRNT data from dengue IgG negative subjects, these results suggest that JEV IgG ELISA test results in dengue-endemic areas should be viewed with caution. More specific laboratory methods, such as JEV PRNT, should be employed where available.
DPT vaccination rate in children ages 1 to 5 years old and associated factors in K'bang District, Gia Lai Province, Viet Nam in 2015

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**Background:** From October 2013 to July 2014, 108 suspected diphtheria cases were reported in 13 out of 14 communes in the K'bang district of Gia Lai province. Seven out of sixteen cases were confirmed positive with diphtheria, including two deaths. The current investigation found that 87% cases had not vaccinated with DPT while the expected coverage of DPT vaccination was 94%. The study aimed to estimate the DPT immunization coverage rate of children 1 to 5 years old and identify associated factors in this district in 2015.

**Methods & Materials:** Using a cross-sectional study design, seven out of fourteen communes were randomly selected. In each commune information regarding vaccination status for 50 children, aged 1 to 5 years old, was collected. This data was used to estimate the overall district vaccination rate using a weighted cluster analysis. Multivariable logistic regression models were applied to identify factors associated with the immunization status of children.

**Results:** 79% of the children surveyed received 3 DPT shots. Based on this study the estimated district vaccination coverage is 81%, 61% were from the Ba Na ethnic group, 87.4% were care for by the communal health center, and 68.7% were vaccinated in that communal health center. 92.3% of the mother or father received the vaccination information from commune health workers. Characteristics associated children receiving full vaccination were their ethnic group (OR = 0.45, 95% CI = 0.22, 0.89); their registration with the communal health center (OR = 0.02, 95% CI = 0.01, 0.06); the education level of mother (OR = 1.62, 95% CI = 1.19, 2.23); their economic status (OR = 1.91, 95% CI = 1.11, 3.29); the parents’ understanding of vaccination (OR = 0.40, 95% CI = 0.24, 0.68).

**Conclusion:** This study shows that a significant gap exists between the observed vaccination coverage (81%) and the goal immunization coverage rate (98%). Groups that need specific attention ethnic minorities and those who are not registered at communal health centers. This study suggests that many parents do not get their children vaccinated because of a lack of understanding and educations campaigns should be introduced to improve vaccination uptake.
Drug-resistant tuberculosis in children less than 5 years old with culture positive mycobacterium tuberculosis

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**Background:** Diagnosis of paediatric tuberculosis remains a challenge due to the difficulty in obtaining samples from children and the low sensitivity of culture confirmation. Drug resistance in TB continues to be a significant challenge in South Africa. Microbiologic confirmation of tuberculosis in children is necessary to exclude drug-resistant tuberculosis in face of the high multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) rates reported in the adult population.

We describe the rates of drug-resistant TB in children less than 5 years old from KwaZulu Natal – a province in South Africa with the highest burden of both TB and HIV disease.

**Methods & Materials:** A retrospective descriptive analysis was done of specimens from children less than 5 years old submitted to TB reference laboratory in KwaZulu-Natal, South Africa. Data was collected from 2012 to 2014. Specimens cultured included respiratory samples, lymph node aspirates, pleural and peritoneal fluid, cerebrospinal fluid, bone and tissue samples.

Cultures were performed using the automated Mycobacterial Growth Indicator Tube 960 system (Becton Dickinson) and identification and susceptibility was confirmed with the line probe MTBDR plus assay (Hain-Life Science). From 2012 the Xpert MTB/RIF was introduced into the diagnostic algorithm for MTB detection and Rifampicin resistance.

**Results:** 903 children were found to have culture-confirmed TB during this 3 year period. Drug susceptibility testing showed susceptible MTB ranging from 71-82 % in the various age groups. Overall the resistance to isoniazid and rifampicin (MDR) rates ranged from 11-16 % with the highest rates found in 2 year old age group. Extensively drug-resistant TB (0-2.1%) was present in all age groups. INH mono-resistance was 3,4% and Rifampicin mono-resistance was 2.8%.

**Conclusion:** High rates of *Mycobacterium tuberculosis* including extensively drug resistant TB were found in children less than one, which indicates the burden of TB infection among woman during childbearing years. INH mono-resistance is of concern as this will not be detected by the current diagnostic algorithm that includes the Xpert MTB/RIF for MTB detection and Rifampicin resistance. Children with INH mono-resistance may benefit from high-dose isoniazid therefore bacteriological confirmation through culture is important in management of childhood TB.
Background: Mycobacterial genome codes for several lipoproteins, and constitute a major component of cell wall. Few of these lipoproteins have been characterized, but function of most of them is yet to be ascertained. Here we establish the role of a previously uncharacterized mycobacterial lipoprotein in intracellular survival through Vitamin D receptor signalling and cathelicidin inhibition.

Methods & Materials: Over-expressing strain of Mycobacterium smegmatis was produced by cloning the lipoprotein gene using shuttle vector pSMT3. The cloned strain was checked for its intracellular survival in peritoneal macrophages isolated from mouse and human monocytes cells by Colony Forming Unit (CFU) assay and Flow cytometry analysis with SYTO 9-PI staining. To elucidate role of Vitamin D receptor signalling in bacterial survival, intracellular survival was checked by exogenous addition of active vitamin D3 hormone 1,25-dihydroxyvitamin D3 (1,25D3). Antimicrobial responses to overexpressing strain were evaluated by checking expression of LL-37 and its upstream signalling molecules by Real-Time PCR. Effect on phagosomal maturation was studied by checking for expression of early and phagosomal markers such as EEA1 and Rab7 respectively in presence and absence of VitD3.

Results: M.smegmatis over-expressing putative lipoprotein was successfully prepared using pSMT3 shuttle vector. Significantly high CFU counts after 48hrs of infection higher intracellular survival in case of lipoprotein over-expressing strain in both mouse and human macrophages. Decreased PI population among intracellular bacterial as compared to vector control corroborated CFU data. Overexpression of Lipoprotein showed TLR2/4 mediated downregulation of Vitamin D Receptor-related gene Cyp27B1 hydroxylase and consequent suppression of cathelicidin expression in human monocytes. Furthermore inhibition of EEA1 (early endosome) and Rab7 (late endosome) in case of lipoprotein over-expressing strain shows that the gene arrests VitD3-PI,3K mediated phagosomal maturation.

Conclusion: The current study suggests that that putative lipoprotein plays an important role in bacterial survival inside host macrophages. It mediates survival by inhibiting VitD3 mediated anti-microbial response mechanism of host cells. It is hoped that the study provides base for further investigation of mycobacterial virulence and survival.
Synthesis, ADME and antimycobacterial studies of a novel series of 2-thiazolylimino-5-arylidene-4-thiazolidinone derivatives
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Background: The emergence of multi-drug resistant and extensively drug-resistant cases of tuberculosis has lead to the search for new structural classes of antituberculosis drugs that can be effective against these strains of *Mycobacterium tuberculosis* (*M. tb*). There are many reports on antimycobacterial screening of compounds containing the 4-thiazolidinone moiety. The 5-arylidene moiety in the 2-heteroarylimino-5-benzylidene-4-thiazolidinone scaffold plays an important role in antimicrobial activity against Gram-positive and Gram-negative bacteria, yeasts and moulds. We have previously reported the synthesis and antimycobacterial activities of 2-thiazolylimino-5-arylidene-4-thiazolidinone derivatives (AIA 2015, 13, 2, in press). In this study a series of 2-thiazolylimino-5-arylidene-4-thiazolidinones were synthesized and evaluated for their *in vitro* antimycobacterial activity against *M. tb* H37Rv.

Methods & Materials: The 2-thiazolylimino-5-arylidene-4-thiazolidinone derivatives were synthesized as reported earlier, and their structures were confirmed on the basis of spectral data and elemental analysis. Qikprop, the ADME prediction program was used in predicting pharmacokinetic properties of the derivatives, which helped in designing and synthesis of novel and more potent analogs. *In vitro* antimycobacterial activity against drug-sensitive *M. tb* H37Rv strain was evaluated and expressed as % inhibitions. Compounds were tested at 6.25 µg/ml concentration in BACTEC-460 TB radiometric system, and Isoniazid and Rifampicin were taken as reference standards.

Results: The synthesis and antimycobacterial activities (% inhibitions) of 2-thiazolylimino-5-arylidene-4-thiazolidinone derivatives are reported. The chemical modifications not only altered the physicochemical properties but also pharmacological activities. The results revealed that most of the compounds exhibited moderate to excellent *in vitro* activity (88-99.7% inhibition) against *M. tb* H37Rv, and it was considerably affected by various substituents on the aromatic ring. Few active derivatives were demonstrating >99% inhibition of *M. tb* H37Rv at 6.25 µg/mL. The activity was considerably affected by various substituents on the aromatic ring of the 4-thiazolidinone, and compounds with di- and tri- substitutions on the aromatic ring were more active than monosubstituted derivatives.

Conclusion: Several compounds were identified as novel and potential lead for design and synthesis of new antimycobacterial agents. These preliminary but encouraging results indicate that 2-thiazolylimino-5-arylidene-4-thiazolidinones are promising scaffolds for design and development of new molecules for antimycobacterial activity. Further structural optimization and identification of molecules are underway in our laboratory.
Antibiotic susceptibility profile of enteric organisms from healthy individuals in a tertiary institution in Nigeria
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**Background:** The world health organization has declared antibiotic resistance as a pandemic which calls for urgent attention. Antibiotic resistance of *Shigella* spp. and *Escherichia coli* isolates from diarrheal patients in Nigeria has been reported to be on the increase (Iwalokun *et al.*, 2001; Yah *et al.*, 2007; Kimang’a, 2012). *M. morganii* had been previously implicated in nosocomial infections, sepsis and other infections (Singla *et al.*, 2010). *M. morganii* has been reported to possess resistance plasmid which is of great importance when implicated in infections. Antibiotic resistance rate among enteric bacteria is becoming alarming and this therefore calls for a constant monitoring of antibiotic resistant organisms in the environment especially among healthy individuals.

**Methods & Materials:** Forty-two stool samples were obtained from healthy individuals in a tertiary institution. Stool samples were obtained in March 2015. These were processed using standard microbiological and bacterial isolates were characterized with Microbat 24E. Antimicrobial susceptibility profile of the isolates was obtained using the disc diffusion method according to Bauer *et al.*, 1966 and NCLS, 2000.

**Results:** Eight-nine bacterial isolates comprising of *Escherichia coli*, *Escherichia fergusonii*, *Enterobacter agglomerans*, *Enterobacter sakazakii*, *Citrobacter youngae*, *Citrobacter freundii*, Enteric group GP59, *Morganella morganii*, *Salmonella enterica* subspecies arizonae (IIIb), *Salmonella enterica* subspecies diarizonae (IV), *Salmonella pullorum*, *Proteus vulgaris*, *Proteus mirabilis*, *Yersinia aldovae*, *Yersinia intermedia*, *Serratia marcescens*, *Serratia liquefaciens*, *Acinetobacter iwoffii*, *Klebsiella ozaena*, *Klebsiella oxytoca*, *Providencia stuartii* and *Hafnia alvei* were isolated and characterized. *Escherichia coli* was the most prevalent (25.8%), followed by *E. agglomerans* (21.3%) with the least prevalent being, *Acinetobacter iwoffii* and *Providencia stuartii* (1.1%). High antibiotic resistance of the isolates to cefuroxime (92.1%), ceftriaxone (92.1%) and ceftazidime (91.0%) was observed, the least resistance was observed towards gentamycin (20.2%). About 80% of the isolates showed multi-drug resistance to commonly prescribed antibiotics.

**Conclusion:** The high antibiotic resistance observed in these bacterial isolates from healthy individuals revealed the need for constant monitoring of antibiotic resistance bacteria and the urgency for sensitization for personal hygiene practices as these may serve as sources of infection within the community.
Socio-economic and demographic impact on malaria prevalence in Akoko South-west of Ondo state, Nigeria
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**Background:** Malaria is an endemic disease prevalent in the tropical and sub tropical region of the world. About 216 million people are still affected by malaria yearly killing about 650,000 people with children under five and pregnant women mostly affected. Many people had died unnecessarily due to malaria due to their inability to procure hospital prescribed drugs especially where free malaria drugs are not available.

**Methods & Materials:** Three basic instruments used for this research were observation, interview and questionnaire. Both primary and secondary data were used for this study. Here data on pregnant women who attend antenatal clinics were collected via well structured questionnaires for the primary data and data on hospital records of malaria episode of pregnant women in the past year was also collected from the hospital archives and their addresses well noted. Two hundred and four pregnant women were involved in the study. The pregnant women were grouped into two based on their age (i.e. 15-35 & 36-45). Socioeconomic and demographic factors such as age, educational, occupational, income and malaria prevalence were considered. Statistical techniques used for the study were ANOVA and Chi-square analysis.

**Results:** Of the 204 pregnant women who administered the questionnaire, 136 had malaria infection. The prevalence of infection among younger age range (15-35 years) was significantly higher (25.89%), than those with middle age group (10.3%). The prevalence of infection was higher among those with secondary education (54.4%) when compared with those with tertiary education (14%). The prevalence of malaria infection was higher among those who engaged in craft work and those without job (22.8% and 22.1% respectively) compared to the civil servants (8%). Based on income per capital the study also revealed that the prevalence of infection was highest among those with lowest income per capital (43.4%) and 5.1% in highest income earners. Secondary data attributed 57.8% of total malaria episode to pregnant women and its mostly due to poverty.

**Conclusion:** This study shows that socioeconomic and demographic factors play a significant role in the prevalence of malaria infection in Nigeria according to the study.
Prevalence of tuberculosis among pregnant women in high burden setting in Sudan using Interferon gamma (IFN-\(\gamma\)) releasing assay (IGRA)
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**Background:** Tuberculosis (TB) is a significant contributor to maternal morbidity and mortality in eastern Sudan.

**Methods & Materials:** A cross sectional hospital-based study carried out in Kassala hospital, Eastern Sudan between January and March 2015 to investigate the prevalence rate of TB and its associated factors during pregnancy using gamma interferon (IFN-\(\gamma\)) release assay (IGRA).

**Results:** Two hundred and forty nine women were approached during the study period and 18.1% (45/249) had confirmed positive for M. tuberculosis infection using gamma interferon (IFN-\(\gamma\)) release assay (IGRA). The sputum test (Acid-fast bacillus - AFB) was found positive in 10 (22.2%) women out of these 45 cases. The mean age, parity and gestational age of the TB patients was 29.6 (4.4), 2.2 (1.2) and 21.9 (8.8) respectively. The vast majority of these patients were of rural residence (72.7%), housewives (91.1%) and illiterate (73.3%). Most of these patients (20, 44.4%) were a symptomatic, more than half (25, 55.6%) gave history of contact with tuberculosis patients, 26.7% (12/45) were vaccinated and 11.1% (5/45) had medical history of diabetes mellitus. In logistic regression model, while age, parity, education, occupation, size of family members, smoking, BCG status and medical history of diabetes mellitus were not associated with tuberculosis during pregnancy, history of contact with TB patients(OR=13.5; CI=5.6-32.5; P=0.000) and rural residence (OR=0.3; CI=0.1-0.7; P=0.006) were significantly correlated to TB in pregnancy.

**Conclusion:** Screening of all pregnant women living in high burden setting of tuberculosis is recommended even in the absence of overt clinical signs of the disease.
APAIDSON program evaluation of the largest private public partnership consortium for HIV/AIDS care and treatment in India

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Background: In India, private institutions were not involved in the activities of National AIDS Control Program (NACP). Andhra Pradesh AIDS Consortium (APAIDSCON) the largest public health partnership a network of 20 medical colleges established to address HIV/AIDS in India was spearheaded by SHARE India and funded in part by United States Centers for Disease Control & Prevention Cooperative agreement # U62/CCU025160-02. 2005 –2010. A program evaluation based on CDC Project Evaluation protocols was undertaken in 2013-14 to evaluate APAIDSCON results.

Methods & Materials: To evaluate HIV/AIDS services 115 patients accessing HIV testing and counselling services and 115 PLHIV from in patient wards were selected at random from 4 randomly selected partnering institutes. Structured questionnaires and in-depth interviews (IDI) and FGDs were used.

Results: 47,260 clients availed HIV testing services from evaluation sites during April 2008 - March 2011. There was a significant increase in proportions of High Risk Groups getting tested (Z score 10.68; p <0.01). There was a significant increase in proportion of PLHIV Outpatients (Z score 2.29; p <0.02) as well as inpatients (Z score 3.41; p <0.01). During April 2008 to March 2011, 90% of eligible PLHIV identified at ICTC were initiated on ART. 118 positive pregnant women had institutional deliveries and 93.3% (110) of the women and 95% (112) of children received nevirapine for prevention of parent to child transmission. 65 infants born to HIV positive mothers were followed up at 18 months and all the 65 children were tested for HIV. Quality of HIV testing at these sites had 100% concordance on EQAS. The overall satisfaction was graded as high by the beneficiaries (N=115). Measures were taken by APAIDSCON to reduce stigma and discrimination in the work place like training and sensitization programs for HCPs, guidelines/protocols implementation to reduce stigma and discrimination, prevention of segregation i.e. no separate bed or special identification marks during admission, staff compensation and encouragement of universal precautions.

Conclusion: Public Private Partnerships like APAIDSCON demonstrate the utility of a basket of services, which have a positive effect on the institutional performance and access to services for PLHIV
Use of contact isolation to prevent spread: Ebola outbreak in a healthworkers base camp, Port Loko District, Sierra Leone, March 2015

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Background: The West African Ebola disease outbreak which started in Guinea in December 2013 has ravaged neighboring countries including Sierra Leone. Health care workers were reported to be the most at risk population. In March 2015, an exposed health worker developed fever and other non-specific symptoms of EVD which prompted investigation to describe its magnitude, identify and ascertain level of risks of contacts and contain the outbreak using contact isolation techniques.

Methods & Materials: We conducted a descriptive study. We conducted active case search and reviewed health records. The case was investigated with real time PCR and all the contacts were identified, line-listed and followed up. We re-defined a contact as any person with no signs and symptoms but had physical contact with the case or body fluids of the case. We collected information on socio-demographic characteristics and categorized contacts according to risk status. Additional information was obtained orally. Data obtained were analyzed using Microsoft Excel statistical software.

Results: The IHP Ebola outbreak generated 2 cases and 51 contacts. Eight (15.7%) of the total contacts were higher risk contacts while 43 (84.3%) were lower risk contacts. Mean age of the contacts was 40.5±5.9 years. One of the two cases was male. Twenty (39.2%) of the 51 contacts were Nurses, 9 (17.6%) clinicians, 2 (3.9%) Anesthesiologists, 3 (5.9) data analysts, 3 (5.9%) epidemiologists, 2 (3.9%) Laboratory scientists, 2 (3.9%) Laboratory Technicians, 2 (3.9%) Medical Interns, 2 (3.9%) Morticians, 3 (5.9%) Public Health Officers, 2 (3.9%) Radiologists and 1 (2.0%) Pharmacist. Six (11.8%) contacts took ill while under observation, and 2 (33.33%) tested positive to the virus when blood samples was subjected to real time PCR. The incubation period was estimated to be 2-6 days with a case fatality rate of 0% despite the high infectivity. The secondary attack rate for the outbreak was 3.9%.

Conclusion: Ebola outbreaks though a deadly infection; early identification and hospitalization of cases, strengthened surveillance, adequate contact tracing and prompt emergency response has proven to be major factors in curtailing further spread.
Outbreak of methanol poisoning in a semi urban community, Ondo state, southern Nigeria, April-May 2015: A descriptive analysis

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**Background:** Patients of methanol poisoning often need intensive care and can result to high mortality. Methanol poisoning with an outbreak potential is uncommon in Nigeria. In April, 2015, we investigated a reported outbreak of methanol poisoning in Ode-Irele, Ondo State. We identified the source(s) and determined risk factors for methanol poisoning in the area.

**Methods & Materials:** We obtained socio-demographic data of suspected cases. We conducted an active case search in the community and health facilities using a semi-structured, interviewer administered questionnaire. Case definition was any person from Ode-Irele, presenting with headache, blurring of vision, and any of blindness, respiratory distress, loss of consciousness, sudden death, with onset of symptoms occurring 24-48 hours prior to 12th April, 2015. Detailed history of chronology of symptoms was elicited and hospital records were also reviewed. We sent samples of urine and informally-produced spirit drinks for laboratory analysis.

**Results:** Of the 39 cases line-listed, 38 (97.4%) were males, with 29 deaths (CFR 74.4%). Most frequently reported symptoms were blindness 29 (82.9%), blurring of vision 28 (82.3%), headache 17 (54.8%). Almost all of the cases were males with majority being farmers and thirty two (94.1%) of the case-patients claimed to have consumed informally-produced spirit drinks, prior to onset of symptoms. Analysed samples of urine and informally produced spirit drinks also revealed toxic methanol concentrations respectively.

**Conclusion:** Ode Irele community experienced an outbreak of methanol poisoning with high fatality. The outbreak was contained through intensive case management and community mobilization. Community health education sessions were held and trainings on lifestyle modification conducted.
Binomics of mosquitos in Anambra State, Nigeria
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**Background:** Mosquito-borne infections constitute major public health challenge in Nigeria. Following indoor residual spraying (IRS) in some communities, there was the need to study species distribution, breeding habitats and infection rates to inform efforts at the elimination of mosquito-borne infections.

**Methods & Materials:** Collection of larval mosquitoes was carried out using appropriate sampling techniques for specific habitats. Adult indoor and outdoor biting mosquitoes were sampled using Pyrethrum Knockdown Collection (PKC) and Human Bait Collection (HBC) techniques, respectively. Blood fed mosquitoes were dissected for infection using the pressing method.

**Results:** 307 mosquito larvae comprising 3 genera and 5 species (*Anopheles gambiae*, *Aedes simpsoni*, *Ae. albopictus*, *Ae. aegypti* and *Culex quinquefasciatus*) were collected from 4 different breeding habitats (ground pools, domestic containers, drainage/gutters and plant axils). 684 indoor mosquitoes comprising *An. gambiae* 39.3%, *Cx. quinquefasciatus* 60.5% and *An. moucheti* 0.2% were collected. 143 outdoor mosquitoes comprising *Ae. aegypti* 72.7%, *Ae. albopictus* 23.0%, *Ae. africanus* 2.8% and *Ae. simpsoni* 1.4% were collected. Zero infection rates were recorded for dissected species.

**Conclusion:** Dissected mosquitoes showed zero infection rates probably due to the recent IRS in the area studied. The 5 species identified are potential vectors of diseases of public health importance and action is needed in manipulating the identified 4 breeding habitats in order to protect community members from mosquito bites and possible transmission of infections.
Hospital Based Surveillance for Radiological Pneumonia in children under 5 years of age in Uttar Pradesh and Bihar: Project protocol and preliminary results

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Background: Pneumonia is responsible for about 2 million deaths in children <5 years, mostly in developing countries like India and within India in the states of Uttar Pradesh (UP) and Bihar. Hence dedicated efforts are required to focus on strategies to reduce pneumonia specific mortality.

Methods & Materials: Aim: To estimate the incidence of radiological pneumonia in children <5 years.

Objectives: Primary objectives are to estimate the annual incidence of radiological pneumonia in children aged 2 – 59 months in districts of Lucknow and Etawah in UP and Patna and Darbhanga in Bihar, residing in pre-specified district as well as to document clinical and demographic characteristics of cases of WHO defined community acquired pneumonia (CAP) with lower chestindrawing (LCI) and severe CAP by establishment of hospital-based surveillance network.

Study design: In a prospective design, hospital-based radiological pneumonia surveillance is being done in Lucknow district and will begin in other 3 from 1.1.16. Cases will be enrolled from network hospitals. Clinical and demographic data will abstracted and chest x-ray (PA) view obtained and archived electronically. An independent panel of radiologists trained in the WHO standard reporting methodology, will interpret x-rays. In Phase I (2015), standard operating procedures were developed and validated as well as web-based data entry software developed by Central Coordinating Unit, King George’s Medical University (KGMU), Lucknow. Thereafter in Phase II surveillance will be initiated in three other districts in addition to Lucknow.

Sample size: Assuming incidence of radiological pneumonia is 3.0/100 child years and for a margin of error of 1.5, incidence of pneumonia in community of 20/100 child years, alpha level of 0.05 and power of 90% when the estimated population of children <5 years in Lucknow district is 750,000; 693 cases have to be included in radiological surveillance study.

Results: Preliminary results will be shared.

Conclusion: Implications: Baseline incidence of radiological pneumonia in high infant mortality states will be estimated which could form the basis of taking evidence based informed decisions for instituting control measures.
A cross sectional study on knowledge and perception about risk factors of selected vector borne diseases among the population of rural field practice areas of KSHEMA, N. Priyadarisini, S. Badiger, N. UdyaKiran, A. K. Shetty.

Background: Malaria, Dengue and other vector-borne diseases (VBDs) are a major public health threats in India. Due to increasing resistance to both drugs and pesticides, there is a need to establish integrated vector management strategies. These strategies should involve local communities in managing the ecosystem to reduce health risks and increase the sustainability of programmes to control VBDs. An important step in disease management is educating the local community regarding VBDs and their risk factors. We assessed community knowledge and perception regarding risk factors of selected vector borne diseases (such as malaria, dengue, filariasis) in two villages in the state of Karnataka, India.

Methods & Materials: A cross sectional study was conducted among 966 adults of Kuthar and Manjanady villages in Mangalore taluk of Dakshina Kannada district in Karnataka. The village sites are attached to field practice areas of K S Hegde Medical Academy. A minimum 5% of total population of the villages were taken using simple random sampling.

Results: Out of 966 adults (69.8% were females and 38.2% were males). The mean age was 40 years. Majority belonged to class IV of Modified BG Prasad Socioeconomic Scale 627(64.9%); 309 (32%) had primary level education. 398(41.2%) of the study population had good knowledge and 568(58.8%) had poor knowledge regarding risk factors of VBDs. Regarding perception 524(54.2%) of participants had average perception, whereas 261(27%) had high perception regarding risk factors of VBDs. The most common perceived risk factors were: Open storage of water(92.4%), Mosquito bite(91.8%), Rainfall(92.2%), Stagnant water(88.8%) and Sleeping outdoors(88.1%). Educated people had higher risk perception than the uneducated (p value <0.05) and females had higher risk perception than males (p value <0.05). When knowledge and perception regarding risk factors of VBDs were associated. Around 96.7% of the population who had high knowledge had average to high risk perception (p value <0.05). A positive correlation (Pearson correlation= 0.55) between the knowledge and perception regarding risk factors of VBDs was seen.

Knowledge about risk factors of vector borne diseases

Relationship between knowledge and perception about risk factors of vector borne diseases

Conclusion: Community knowledge plays a vital role in perceiving the risk factors of VBDs. Future public health strategies must focus on community health education to eliminate VBDs in India.
Meningococcal carriage in University freshmen in Kashmir, North India

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Background: Meningococcal disease is endemic in many parts of the world, with relatively large-scale outbreaks occurring in many countries. High density populations like dormitory students and military recruits are considered to be high risk groups to contract meningococcal disease. Indian data on meningococcal disease are sparse and limited to the studies undertaken during or immediately after suspected outbreaks.

Methods & Materials: In a cross sectional design, 274 consenting healthy college freshmen (age 17-19 years, median 17; 48 female) were approached within 7 days of joining the hostel, where they would share their rooms with 2/3 more students. Demographic data and any high risk behavior was recorded on a predefined proforma. Charcoal impregnated nasopharyngeal swabs were obtained, transported to the laboratory within 2-3hrs, plated directly onto Thayer Martin medium and incubated at 37°C with 5-10% CO2 for 24 hours. In case of no growth the plates were incubated for another 24 hours and examined subsequently for oxidase positive Gram negative diplococcic suggestive of Neisseria spp. The isolates were sub cultured, DNA isolated and Sanger sequencing performed on the amplified PCR product. Blast n was performed at different score ad E-value parameters for all positive sequences against the whole NCBI nr/nt data base. Molecular phylogentic analysis with various serogroups of N.meningitides was performed by using MEGA 6 software package.

Results: Of the 274 nasopharyngeal swabs, 10 (3.6%) grew Neisseria. DNA isolation and Sanger sequencing was performed on the amplified PCR product and sequence analysis was carried out. Blast analysis of all sequenced samples was performed against the whole NCBI-nr/nt database and within the Dataset. On molecular testing and sequence analysis, 4 of the samples were found to be N. meningitidis whereas one had close similarity to N. meningitidis. Only 2 students reported history of intimate kissing in the past 2 weeks and one had a history of using antibiotics. The isolates on Blast and molecular phylogeny analysis bore homology to serogroup ’B (Fig 1).

Evolutionary relationship of N.meningitidis with different published serogroups

Conclusion: Neisseria meningitides is seen in college freshmen and the potential implications for spread in close shared settings call for appropriate infection control measures.

NCBI Blast hits of Sequence ID (Skimsmen0010) against whole NCBI (nr/nt) Data base.
An evaluation of a 24 hour malaria mobile case reporting system compared to the paper-based case reporting system in South Africa, 2015

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**Background:** South Africa is pursuing a malaria elimination initiative, with the goal of zero local transmission by the year 2018. The South African National Department of Health has developed a 24 Hour Electronic Reporting System that is expected to decrease the time required for malaria cases to be reported from the health facility level, to the district, provincial and national levels. This will facilitate case investigation within 48 hours and response within 72 hours upon/of diagnosis, the objective of our study was to compare timeliness of data input between the paper-based and the 24-hour reporting system and to assess the acceptability of end users to use the new 24-hour reporting system.

**Methods & Materials:** This is a prospective descriptive study, using a questionnaire with open and close ended questions which was developed using Epi Info 7. We compared 148 cases of the current reporting paper-based system and the 24-hour reporting system (Unstructured supplementary service data-USSD platform). We analysed the responses using STATA version 13.

**Results:** The preliminary findings from the study show that the mean time difference in days from date of diagnosis to the case being captured was one day for the 24-hour reporting system and seven days for the current paper-based system. The mean time difference in days for case investigation to occur using the paper based system is 5.4 days. All 120 health care facility workers interviewed found the system to be simple to use and were willing to use the system to report malaria cases.

**Conclusion:** The 24-hour reporting system has decreased the time required for malaria cases to be notified from the healthcare facility to the district, provincial and national levels. The next step will be to assess whether this system will improve investigation timeliness (48 hours from diagnosis) and response (72 hours) for those sub-districts that have an annual parasite rate of <0.1/1000 population at risk.
Emergence of multidrug-resistance in tuberculosis cases: Role of risk factors

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Background: In 2013, 6.1 million tuberculosis (TB) cases were reported to WHO. Of these, 5.7 million were people newly diagnosed and another 0.4 million were already on treatment. Globally, 3.5% of new and 20.5% of previously treated TB cases were estimated to have had multidrug resistant (MDR) - TB. The previous treatment for TB is the strongest risk factor for development of MDR-TB. The current scenario states that even treatment naïve patients are also at risk.

Methods & Materials: The study was a prospective observational study aimed to identify the group at high risk was carried out over a period of 9 months in govt. hospitals in the Telangana region. The patients were enrolled based on the MDR-TB suspect criteria: failure, S+ at 4th month (retreatment case), contact of known MDR-TB case, S+ at diagnosis (retreatment case), any follow up S+, S- at diagnosis (retreatment case), and HIV-TB case. Results were analyzed using fisher’s exact test.

Results: Among 2033 suspected MDR-TB patients, 453 (22%) showed resistance to isoniazid/rifampicin or isoniazid and rifampicin. Out of 453, 199 (43%) were resistant to Isoniazid, 173 (38%) were resistant to both Isoniazid and rifampicin, and 81 (17%) were resistant to rifampicin. Patients aging <45 years (76%) and >45 years (23%) were found to be at equal risk (p=0.085). Analyzing 453 MDR-TB cases, males (78%) were found to be at higher risk to develop MDR-TB (p=0.002). But if we consider the whole suspect population (2033), out of 1641 male suspected cases, 355 (21%) were diagnosed with MDR-TB and out of 392 female suspected cases, 98 (25%) were diagnosed with MDR-TB. This clearly states that both genders are at equal risk. Among 453 suspected cases, S+ at diagnosis-retreatment case (291) were found to be at high risk followed by any follow up S+ (84) and HIV TB case (32).

Conclusion: The study concluded that each and every suspect individual is at equal risk to develop MDR-TB. The high level of significance in risk of developing MDR-TB directs this study further to check for the strategies to reduce the rate of TB and MDR.
Waning maternal measles antibodies in infants

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Background: Measles continues to be a major cause globally of childhood morbidity and mortality and was responsible for 145,700 deaths worldwide in 2013. Many countries have now adopted measles elimination targets including China, which was originally slated for elimination in 2012 as part of the WHO’s Western Pacific Region measles control plan. This elimination goal was not met despite China’s intensive control efforts over several years, and sustained levels of transmission continue to characterize measles epidemiology there. In order to better understand measles susceptibility in Tianjin, China, the University of Michigan School of Public Health and the Tianjin Centers for Diseases Control and Prevention collaborated on a large, population-based, cross-sectional, seroprevalence and case control study conducted throughout the municipality with a special focus on documenting the serostatus of mother-infants pairs. Infants play a major role in ongoing disease transmission in China even though measles vaccination is provided free at age 8 months.

Methods & Materials: We interviewed and drew dried blood spots (DBS) from a systematic random sample of 2818 people from every Tianjin district, including 809 mother/infant pairs. The DBS were tested for measles IgG to determine measles susceptibility.

Results: While the majority of mothers (81.5%) tested IgG positive only 16.3% of all infants less than 8 months of age tested IgG positive. Among infants one month of age or less, only 40.3% were IgG positive. That level of IgG positivity dropped to 12.3% at age 3 months, 6% at age 5 months; no infants were IgG positive by age 7 months. There was no significant difference in in the percentage of infants with a measles positive IgG result by birthweight, rural vs urban district, or mother’s vaccination status, education and age group. Infants of mother’s with a history of measles infection and of mothers who are non-residents had a higher percentage of measles IgG positivity.

Conclusion: Although waning maternal measles antibodies in infants is well-documented, we found this may be occurring at younger ages than previously documented. This could have major implications for measles elimination programs in China and globally requiring new strategies to target transmission in this age group.
Spatial analysis of patients with multi-drug resistant pulmonary tuberculosis between 2009 and 2012 in Eastern China

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**Background:** China has the second highest tuberculosis (TB) burden worldwide, with approximately 1 million new cases per year. Moreover, multidrug-resistant tuberculosis (MDR-TB) poses a major threat to TB control in China. In 2008, 5.7% of newly diagnosed and 25.6% of previously treated TB patients in China had MDR-TB. This study aims to analyze the spatial distribution of patients with multi-drug resistant pulmonary tuberculosis in Zhejiang Province, eastern China, to identify hotspot that may be subject to clustering of transmission, and provide a theoretical basis for the further study of the risk factors of tuberculosis and its prevention and control strategy.

**Methods & Materials:** We collected the information related to notified cases of multi-drug resistant pulmonary tuberculosis patients from at county level in Zhejiang Province between 2009 and 2012, and analyzed the survey data by ArcGIS 10.0 software using geographic information system spatial analysis method.

**Results:** During the study period, there were a total of 647 patients with multi-drug resistant pulmonary tuberculosis of all 90 counties in Zhejiang Province, overall space distribution showed a trend of central and southwestern more gathering. Spatial clustering analysis of the cases in 2009 identified 2 clusters, the first cluster included two urban areas, the other included rural sites; spatial clustering analysis of the cases in 2010 identified 1 cluster, included 5 counties/districts; spatial clustering analysis of the cases in 2011 identified 2 clusters, the first cluster included 5 counties/districts, the other included 2 counties from mountain areas; spatial clustering analysis of the cases in 2012 identified 2 clusters, the first cluster included two urban districts in the capital of Zhejiang Province, the other included 5 counties in relatively developed areas.
Conclusion: There was a gathering phenomenon of patients with multi-drug resistant pulmonary tuberculosis in the central and southwestern of Zhejiang province between 2009 and 2012, the notifications were not randomly distributed in space, clusters did exist in Zhejiang province, and the clusters most likely existed in the region of Quzhou, Southwestern of Zhejiang, and the region of Hangzhou, central Zhejiang.
Prevalence and characterization of group B streptococcus among pregnant women at a tertiary hospital in South Africa

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Background: Group B Streptococcus (GBS) is a significant cause of perinatal and neonatal disease. Maternal screening for GBS colonization followed by intrapartum antibiotic prophylaxis is effective in reducing invasive GBS disease in newborns. In this study we determined the prevalence of maternal GBS colonization including antibiotic susceptibility patterns and serotype distribution of GBS among pregnant women at a tertiary-level hospital in South Africa.

Methods & Materials: Vagino-rectal swabs were collected from 284 pregnant women between 26-37 weeks gestation from November 2013 to May 2014. Swabs were cultured onto selective Granada medium and incubated at 35°C under anaerobic conditions for 18-24hrs. Characteristic orange colonies on of GBS on Granada medium were confirmed by Streptex agglutination. Antimicrobial susceptibility was tested against penicillin, erythromycin, clindamycin, vancomycin and levofloxacin by Etest using CSLI guidelines. Serotyping was performed by latex agglutination.

Results: Seventy-two (25%) pregnant women were colonized with GBS. All isolates were susceptible to penicillin (MIC₉₀ = 0.125mg/l), however, reduced MIC values (0.25mg/l, 0.7mg/l and 1 mg/l) were observed in three isolates. Reduced susceptibility to erythromycin (17%) and clindamycin (6%) was also observed. All isolates were susceptible to vancomycin and levofloxacin. The most common serotypes were Ia (54%), III (19%), and V (17%).

Conclusion: We report a high prevalence of GBS colonization amongst pregnant women. Although penicillin remains the drug of choice for treating GBS, active surveillance should be encouraged to monitor susceptibility trends in order to detect emergence of resistance. Erythromycin and clindamycin should only be used in penicillin intolerant cases after susceptibility results are available. Vancomycin or levofloxacin may be considered in a setting of high levels of macrolide resistance. Serotype Ia was found to be the commonest serotype in this study. This is consistent with local and international findings.
Frequency of cutaneous leishmaniasis among patients referred to the Center for Disease Control in Kuhgiloyeh and Boyerahmad province 2009-2013

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Background: Leishmaniasis has an annual incidence of 1.5 -2 million new cases ,and is endemic in 88 countries throughout the world. About 90% of cases of cutaneous leishmaniasis (CL) are reported from seven countries including Iran.

Methods & Materials: Data for this descriptive-analytical study were collected by means of a questionnaire. Most of the CL cases were referred to the Center for Disease Control after diagnosis by direct smear. In order to review the epidemiology of CL in Kuhgiloyeh and Boyerahmad, Iran, factors such as incidence, ulcer types (dry or wet), age, and sex distribution, occupation of patients, and the clinical spectrum of disease were evaluated during a cross-sectional & retrospective study.

Results: 289 patients with confirmed CL, who referred to the urban and rural health centers of Kuhgiloyeh and Boyerahmad province from 2009 to 2013 were evaluated. The highest prevalence of CL was observed in Dehdasht District (50.9%). Although CL was prevalent in both men and women, it had higher incidence in men (54.7%). The majority of patients (44.2%) aged 0-20 yr old. Most lesions were in face (33.9%). Most patients had single lesion on their bodies. Among all occupations, the highest prevalence of CL was detected in students (28.7%). Most common form of lesions were wet (42.6%).

Conclusion: fortunately, the results showed that the prevalence of CL has been low in Kuhgiloyeh and Boyerahmad province in compare to its neighbor provinces such as Fars and Isfahan. Geographical conditions of this province such as high altitude climate, low mean annual temperature and proper disposal of wastes, maybe reasons for low prevalence of cutaneous leishmaniasis in this province.
Assessment of uptake of intermittent preventive therapy for malaria in pregnancy following a health facility based training approach in Akwa Ibom state, Nigeria.

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Background: The Nigeria Demography and Health Survey 2013 reported only 11.6% of pregnant women attending antenatal care (ANC) received Intermittent Preventive Therapy (IPTp) with Sulphadoxine-Pyrimethamine (SP) to prevent malaria. The United States President’s Malaria Initiative funds the Malaria Action Program for States (MAPS) project to implement malaria interventions in selected states including Akwa Ibom State in Nigeria. Previous training of ANC providers on management of malaria in pregnancy in clusters where health workers were selected by their supervisors for the training outside the facility did not yield much behavior change. A change in training strategy to facility based training (FBT) was undertaken. This study assessed uptake of IPTp among ANC attendees following FBT.

Methods & Materials: A before and after study of uptake of 2 doses of IPTp was conducted in nine health facilities (HFs) selected based on history of high volume of ANC attendance and availability of SP. Two-day training on management of malaria in pregnancy was conducted in each HF using participatory and adult learning principles with national training materials including proper documentation and reporting on national registers. Total of 252 health workers were trained between May and August 2015. Data on mean percentage of ANC attendees who received 1st and 2nd doses of IPTp six months before the training were collected from the District Health Information System and compared with a month after the training. Data was analyzed using SPSS v.20 and paired t-test was used to test level of significance at 95% CI.

Results: All the selected HFs successfully implemented IPTp administration as directly observed treatment. Within one month of intervention, documentation using the national registers improved. Mean IPT1 and IPT2 uptake increased significantly from 31.2% to 70.8% (p<0.05), and from 21.6% to 37.0% (p<0.05) respectively.

Conclusion: FBT approach in building capacity of health workers appears to be an effective method in increasing uptake and documentation of malaria intervention among pregnant women in Akwa Ibom. Scale up of this strategy might rapidly increase uptake of IPTp and other malaria interventions. There is need for further studies to compare the effectiveness of other training approaches.
To evaluate the level of oxidative and antioxidative parameters and its relationship with clinical symptoms in women with Primary Fibromyalgia Syndrome

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Background: Primary Fibromyalgia Syndrome (PFMS) is a common chronic pain syndrome with an unknown etiology. Increased oxidative stress results from an imbalance between products of oxidation and antioxidant defenses. There are several clinical conditions associated with increased oxidative stress, but novel data suggest a relationship between oxidative stress and pain perception. Furthermore, there is little information available in scientific literature about oxidative and antioxidative parameters in PFMS. In the present study we examined the involvement of oxidative and antioxidative parameters in women with PFMS and also evaluated its correlation with the severity of its symptoms.

Methods & Materials: Oxidative stress was determined by measuring the levels of Lipid Peroxides (LPO) and Protein carbonyl in plasma and antioxidative parameters like catalase, Glutathione peroxidase (GPx) and Glutathione Reductase (GR) in lysate in 60 female patients satisfying American College of Rheumatology (ACR) criteria for FMS and 60 healthy females without PFMS. Clinical parameters of PFMS were evaluated by Fibromyalgia Impact Questionnaire Revised (FIQR).

Results: Concentrations of catalase (p<0.01), GR (p<0.01) and GPx (p<0.01) were significantly lower in patients with PFMS than in controls, and levels of oxidative stress parameters, LPO (p<0.01) and Protein carbonyl (p<0.01) were significantly higher in patients than in controls. A significant positive correlation was found between LPO and clinical symptoms of PFMS among patients group. Furthermore, a significant positive correlation was also found between Protein carbonyl and clinical symptoms of PFMS among patients group than in control group.

Conclusion: The present results indicate that women with PFMS are exposed to oxidative stress and this increased oxidative stress may play a role in the etiopathogenesis of the disease. Moreover, our results also show that increased oxidative stress parameters are more strongly associated with PFMS symptoms.
Hepatitis A outbreak due to contaminated public water in Tiruchirappalli Corporation, Tamil Nadu, India, 2015
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Background: Hepatitis A (HAV) outbreaks are due to faecal contaminated water or inadequately treated water. On 9 October 2015, Kemps stone area (population=665) of Tiruchirappalli, reported 42 cases of jaundice, all aged less than 20 years. We investigated to identify risk factors and propose recommendations.

Methods & Materials: We defined a case as acute onset of jaundice in age less than 20 years in kemps stone area between 1st July and 12th October, 2015. We conducted a retrospective cohort study. We interviewed for socio-demographic characteristics, food and water sources. We calculated relative risk (RR) and 95% confidence interval (CI). We also calculated attributable risk (AR) and population attributable risk (PAR). We collected 6 sera for testing for leptospires, HAV and Hepatitis E. We collected 3 water samples from old pipeline and 3 water samples from new pipeline.

Results: The population aged below 20 years was 214. We identified 42 case-patients (attack rate:20%(42/214), no deaths) between 13th August and 9th October. There was clustering of cases in 1st to 5th lanes. Sixty two percent [26/42] of cases were reported from 4th to 5th lane, while no cases were reported from 6th to 9th lane. Attack rates were almost equal among females {19%(21/112)} and males{21%(21/102)}. Median age was 11 years. First to fifth lanes had water supply from old pipeline connection, while other lanes had new pipeline connection. Using public water from old pipeline for drinking (RR=3.2, 95% CI=1.9-5.2, AR%=68%, PAR=24%) and for cooking (RR=19, 95%CI=8.9-39.4, AR%=95%, PAR%=79%) were significant risk factors. Using toilet for defecation (RR=0.2, 95%CI=0.13-0.38) and washing hands with soap after defecation (RR=0.5, 95% CI=0.3-0.8) were protective. There was leakage in old pipeline. Overhead tank water was never chlorinated. All sera were positive for HAV. Water samples from old pipeline were non-potable.

Conclusion: This HAV outbreak in kemps stone area was due to using water from old pipeline for drinking and cooking. We recommend repair of old pipeline, chlorination of overhand tank water and periodic water testing. We also recommend creating awareness on avoiding open air defecation and promote hand washing after defecation.
Effect of behaviour change-intervention on hand washing practices and knowledge about hand washing among school students, Perambalur district, Tamil Nadu, India, 2014-2015

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Background: Diarrhoeal diseases among school children could be prevented by proper hand washing practices. Health education interventions will help in improving hand washing practices among school children. We conducted behavioural change intervention among middle school students, to compare adequate hand washing before and after intervention in rural area of Perambalur district, Tamilnadu, India.

Methods & Materials: We did intervention study between November 2014 and April 2015 among 6th to 8th grade students in Ammapalayam block. We recruited 200 students, assuming proportion of hand washing with soap among the children is 17.5% and expecting proportion of hand washing with soap among the same children about 30%, after intervention, with one tail, level of significance of 5%, 90% power and 10% loss to follow up. We selected students through simple random sampling as matched pair. The intervention included oral and poster presentations, demonstration and competition on proper washing procedures to all students at first visit during the baseline survey. Outcomes were collected by semi-structured questionnaire at base line and at two follow up visits (one month and two months after the intervention). We compared the proportions before and after intervention by Mc-Nemars chi-square test.

Results: At one month, proportion of adequate hand washing improved from 0 % at baseline to 7% with Mc-Nemars chi-square=13.07, P-value=<0.001. Knowledge about importance of hand washing improved from 45% to 61% with Mc-Nemars chi-square=16, P-value<0.001. Knowledge about 6-steps in hand washing improved from 0% to 78.5% with Mc-Nemars chi-square=156, P-Value<0.001. At two month, proportion of adequate hand washing was improved to 5.5% with Mc-Nemars chi-square=10.08, P-Value<0.001. Knowledge about importance of hand washing was improved to 61 % with Mc-Nemars chi-square= 138.69, P-Value<0.001 and knowledge about 6-steps in hand washing improved to 86 % with Mc-Nemars chi-square= 171, P-Value<0.001.

Conclusion: After intervention, there was an improvement in adequate hand washing, knowledge about importance of hand washing and knowledge about steps of hand washing at the end of one and two months. We recommend that same intervention may be done in schools to improve the knowledge and hand washing practices among school children.
Polio eradication initiatives, a critical data analysis, District Khairpur, Sindh Pakistan, 2014
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**Background:** By the end of 2014 Polio outbreaks were reported in 04 bordering districts of Khairpur. Pre, process and post campaign prime indicators were reviewed in the light of National Emergency Action Plan (NEAP) strategy for Polio Eradication initiatives (PEI) to find and fill the gaps through appropriate recommendations to minimize the risk of virus circulation in 2015.

**Methods & Materials:** A descriptive study on record review of PEI was conducted from 5th to 10th January 2015 at Polio Control Room (PCR) Khairpur. Data of all 75 Union councils (UCs) gathered and analyzed by the year 2014 on NEAP indicators.

**Results:** For targeting children aged <5 years a total of 11 Supplementary Immunization Activities (SIAs) were conducted.

In pre-campaigns, 18(24%) UCs were identified in deferment criteria (<80%) on indicator of minimum one female member in a mobile team. Mean participation of local government in Union Council Polio Eradication Committees (UPEC) stacked 91% (ranges 83%-96%).

For PEI trainings, a mean of 2% (95%-100%) teams were found absent in trainings however an average of 18% (15%-23%) microplans (n=29%) were field validated by district staff.

In process campaigns, a Mean of 6% (1%-11%) teams found in malpractices neither paying revisits to cover not available children nor recording for catch-up. Moreover 12% (ranges=7%-19%) team supervisors did not monitored their teams. An average of 4000 (1996-5956) recorded missed children were never tracked for immunization.

Post campaigns were evaluated by taking Market surveys showed 2% (96%-99%) children were without finger marking, meanwhile in 04 campaigns LQAS lots were found to be in medium band. although no one lot rejected.

**Conclusion:** Overall performance indicators were below the NEAP requirements. PCRs may be established at UC levels for close coordination by involving local female community volunteers for additional support in catch-up days to track and immunize missed children.
Transporting snakebite victims to appropriate health facility to save lives through emergency ambulance service in India

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Background: Background:
Out of 216 species of snakes in India, 52 are known to be venomous, the big four venomous snakes are Cobra, Krait, Russell’s viper, and Saw scaled viper. In India 15000 to 20000 deaths are reported every year due to snake bites.

Methods & Materials: Methods and Materials:
Analysis of records was done for the year 2014. Source: Server Query Language data from GVK EMRI operating states. Data analysis was done using MS Excel. Victims who complained of Snake Bite and decided to avail or use the Emergency Ambulance Service of GVK EMRI through toll free number 108 emergency services for the period of 12 months (January to December 2014) from GVK EMRI 10 States and 2 Union Territories.

Results: Results:
Total 29,461 snake bite cases were enrolled in this study period. Of these 28436 (97%) used and 1025(3%) did not use108 transportation. 26810 (94%) were total hospital admitted and 1626 (6%) were assessed to not require the transportation service-of these 107 cases had expired before the EMS arrival, 22 were given first aid, 69 cases were expired on reaching to the hospital. Type of hospitals patients transported and admitted to: Government 23996, Private 2614 and Trust 200. Overall mean response time was 00:22:56, on scene 00:10:06, scene to hospital arrival 00:47:02 (hh:mm:ss). Out of 26810 admitted cases 8652 completed 48 hours follow up: 6149-All right and discharged from the hospital, 1893 -Stable, out of danger but still in hospital, 9 -Condition is still critical-in hospital, 408- had expired after 48 hours of transportation and status could not be stated in 183 cases (EMS 108 activated by bystander).

Conclusion: Conclusion:
The GVK EMRI ambulances that are fully equipped with all life saving equipments and drugs including anti snake venom are able to save lives in critical condition of snakebite victims while they call on toll free number 108 in 16 states of India. This model needs to be replicated in other parts of the country and also in high snakebite incident countries of Asia and Africa.

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Health status of industrial workforce in district Lahore Pakistan
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**Background:** Industrial labour is an important population section as it is actively engaged in the industry of the nation. Labour work force and health have bi-directional relationship, as hazardous work can produce negative effect on health in terms of injury and disease. Since sickness and absenteeism is an indicator to measure the health status of Labour workforce as well as an important indicator to measure the functioning of the industrial establishments; therefore, it has been focused to describe the distribution of their health status in this project.

**Methods & Materials:**

**Study Design:** Descriptive epidemiological cross sectional study

**Settings:** Nawaz Sharif Social Security Hospital, Lahore

**Methods**

**Results:** The average age of patients was 36.5 + 10.5 years, which is the productive age group. Out of a total of 87,278 labour patients 83160 (95.28%) were male and 4118 (4.72%) were female. Among the patients 22046 (25.26%) were recorded with infectious diseases and 65232 (74.74%) were recorded as non-infectious diseases. Among infectious diseases, the most frequent conditions with a ranking in the descending order was gastro-intestinal disorders with a number of 10719 (48.62%), tuberculosis & chest infection were 7497 (34.01%) followed by 3699 (16.78%) suffering with skin diseases, while 123 (0.56%) were of hepatitis-C virus (HCV) and the least with hepatitis-B i.e. 08 (0.04%).

Among non-infectious diseases the most common patients reported were 16902 (25.94%) with cardiovascular diseases followed by 10306 (15.80%) of orthopedics, 7439 (11.40%) of urology, 5422 (8.31%) of diabetes and 5047 (7.74%) of various eye diseases.

**Conclusion:** The number of absenteeism due to tuberculosis & pulmonary disorders were statistically higher than number of absenteeism due to other diseases (P <0.001). This magnitude of problem would double the burden on exchequer of the organization concerned with the quality of healthcare services at all levels and improvement in it. The health status of labour workforce affects the economic productivity of the nation’s wealth, therefore, comprehensive health & labour policy is needed to address the health issues of the labour workforce which is the need of the hour, so as to achieve the maximum outcome in a decent work environment.
Bacteriological profile of street vended food

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**Background:** Street foods are tasty, have a local flavour and comparatively cheap. They provide with accessible source of food to all classes of people and maintain the food supply chain of different populations. Globalization, tourism and growth in population have instigated rapid change in food demands, increasing the necessity of public awareness towards the safety of street vended food. The outbreaks of food and waterborne diseases remain as a threat in many parts of the world with microbiological contamination being one of the major reasons leading to infections, serious disorders and long term complications.

**Objectives:** To study the bacteriological profile of street vended food and to elicit the hygienic habits adopted by street vendors and food handlers in selected areas of an urban city.

**Methods & Materials:** A cross sectional observational study was carried out after an approval from institutional ethics committee. Five busy areas of the city were selected by random method. Seventy food and water samples were collected from Platform vendors, Cart vendors and Hawkers. Preparation of food, storage, handling and distribution were documented. Standard methods were adopted for microbiological sampling, transportation, processing and Antibiotic sensitivity testing. A self-assessed questionnaire survey was carried out to evaluate the hygienic practices of the street vendors and all the data was analyzed statistically.

**Results:** Bacterial isolates such as, Salmonella typhimurium(1) and multi drug resistant bacterial isolates of Shigella dysenteriae(2), Vibrio cholerae(2), Escherichia coli(3) and Staphylococcus aureus(2) from different type of food samples more, from the food prepared by the vendors were isolated. Hygienic habits of the street vendors were far from satisfactory.

**Conclusion:** Environmental status in and around the vicinity, improper practices followed by the vendors and bacterial isolates from the food samples are likely to predispose or precipitate outbreaks of food-borne diseases. There is an urgent need for surveillance and active implementation of hygienic food preparation, handling and storage practices by the Local Health Authorities so as to protect the community from epidemics.
The impact of short educational messages in motivating community-dwelling seniors to receive influenza and pneumococcal vaccines

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Background: Influenza and pneumococcal vaccine uptake rates have been consistently low among community-dwelling seniors in Singapore. Our Institute set up a vaccination education booth at a community-based health event for seniors, with the aims of assessing the knowledge, attitudes, practices and behaviours (KAPBs) of seniors towards influenza and pneumococcal vaccines, and evaluating the effectiveness of short educational messages in motivating seniors to receive vaccination.

Methods & Materials: Participating staff were pre-briefed on a standardised process to engage seniors, consisting of three components: a short KAPB survey on influenza and pneumococcal vaccines; an educational brief providing basic information about the vaccines, including their purpose, cost and availability; and an invitation to receive vaccination at the senior’s local healthcare provider. Results of our engagement were collated and analysed. Multiple logistic regression models were applied to determine factors associated with willingness to get vaccinated.

Results: A total of 124 seniors were engaged. The median age was 70 years (IQR 63-76 years). Majority of seniors were female (83.9%) and of Chinese ethnicity (91.9%). 85 (68.5%) were aware of the existence of influenza vaccine, but only 36 (29.0%) had ever received it. 31 (25.0%) were aware of pneumococcal vaccine, but only 10 (8.1%) had ever received it. 72 (58.1%) seniors could state at least one health benefit of vaccination. Following our educational brief, 82 (66.1%) stated they would advise their friends to receive influenza and pneumococcal vaccines, and 86 (69.4%) stated willingness to receive vaccination. 55 (66.2%) out of 83 seniors aged ≥65 years had never received influenza or pneumococcal vaccine before, but 35 of them (63.6%) were willing to after our education brief. In seniors ≥65 years, being able to name at least one health benefit of vaccination was significantly associated with willingness to be vaccinated, after adjusting for gender and history of receiving vaccines (Adjusted OR = 3.26, 95%CI 1.20-8.85; p=0.02).

Conclusion: Short educational messages may serve as useful cues to action to motivate seniors to receive vaccination. This is especially among those with prior knowledge of the benefits of vaccination, where supplementary information (such as vaccination schedule, cost, and the means of accessing services) may facilitate vaccine uptake.
Characterization of antibiotic resistance in escherichia coli isolates from abattoir drains

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**Background:** The increase in antimicrobial resistance among pathogenic bacteria has emerged as an important risk in public health and in human medicine. This may lead to significant health risk to humans with records of disease outbreaks. The aim of this study was to characterize antibiotics resistance of *Escherichia coli* strains from abattoir effluents.

**Methods & Materials:** A total of 75 *Escherichia coli* isolates were isolated from abattoir effluents using standard culture-based, biochemical reactions and polymerase chain reaction (PCR) identification of the suspected colonies. The disc diffusion technique was used to screen for antimicrobial susceptibility against fifteen different antibiotics. The presence of class 1 integrons in each *Escherichia coli* strains was assessed using 3′-CS and 5′-CS regions specific primers. The *sul1*, *sul2*, and *sul3* genes in all sulfonamide resistant isolates were investigated by PCR using primers specific.

**Results:** All the isolates were multi-resistance, defined as resistant to at least four different antibiotics with multiple antibiotic resistance (MAR) indexes ranging from 0.26 to 0.93, signifying that the isolates of high antimicrobial usage origin. Plasmid DNAs were found in 80% of the strains analyzed harbored plasmid DNA, size from 1.2 to 81.5 kb, separating the isolates into 8 different plasmid profiles were observed. The mechanism by which *Escherichia coli* have accumulated antibiotic resistance determinates is of interest. All strains showed resistance against sulfonamides. The presence of class 1 integrons was determined for all tested *Escherichia coli* strains. The relationship of sulfonamide resistance genes with integrons, it was revealed that *Escherichia coli* strains harbored class I integrons with variable regions. Antibiotic resistant *Escherichia coli* could be a major risk to public health as a significant reservoir of encoding antibiotic resistance genes that can be transferred intra or interspecies.

**Conclusion:** The increase of antimicrobial resistance signatures necessitates for adequate sanitation and proper surveillance programs towards monitoring of antimicrobial resistance determinants in abattoir environment.
Detection of clusters and geographical hotspot for Lassa fever in Edo Central Senatorial district of Nigeria: A step into a nation-wide mapping of Lassa fever

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**Background:** Lassa fever is a viral hemorrhagic fever which occurs mainly in Nigeria and other African countries with high mortality during epidemics. Surveillance data shows that Edo Central Senatorial District of Nigeria has the highest number of cases recorded since 1969 when the disease was first discovered in Nigeria. Despite this, there is yet to be a detection of geographical clusters and hotspots of the disease since the first outbreak in 1969.

The objective of the study was to detect the geographical clusters and hotspots of Lassa fever in Edo Central District in order to develop a template for nation-wide mapping and sero prevalence study of Lassa fever and other related viral hemorrhagic fever in Nigeria and in West Africa.

**Methods & Materials:** Using a cross sectional study design, dataset of 213 cases of Lassa fever in Edo Central Senatorial District from 2008-2013 were visualized in space, queried and interpreted using ArcGIS 10.2, a Geographical Information System (GIS) software. Anselin Local Moran’s and Gertis-ord Gi* advanced geostatistical tools were employed in determining geographical clusters and hotspots of Lassa fever.

**Results:** The median age of Lassa fever patients was 30 years (IQR = 29 years). Statistical significant clusters (p < 0.05) and hot spots (p = 0.05) of all reported cases from 2008-2013 occurred in Ekpoma town, Esan West Local Government of Edo State, Nigeria. The hotspot of Lassa fever is a few meters away from Ambrose Alli University, Ujemen, Ekpoma. Clusters of Lassa fever are found at several locations within Ekpoma and Irrua.

**Conclusion:** Clusters and Hotspots of Lassa fever cases in Edo Central Senatorial District recorded from 2008 through 2013 were found in Ekpoma town. Based on the findings, both Local and regional and International disease control teams can now develop a national surveillance and control strategy to contain the outbreaks of Lassa fever and other related viral hemorrhagic fevers in Nigeria and other affected countries.
Background: Hepatitis D virus or delta virus (HDV) is a small, defective RNA virus that can infect only individuals who have hepatitis B virus which acts as the carrier host. The prevalence of HDV antibodies in Pakistani hepatitis B surface antigen (HBsAg) positive individuals is approximately 16.6%. So there is a pool of at least 800,000 anti-HDV positive HBsAg positive individuals in the country. Although the prevalence of hepatitis virus infections in Pakistan is still unknown, limited data indicate that the exposure rate to HBV is 35-38% with 4% being carriers and 32% having anti-HBV surface antibodies through natural conversion. Studies in Pakistan have shown that the prevalence rate of HDV is 4.8-14% for, and that it is continuously increasing. Hence there is an urgent need to create awareness about the prevalence of both hepatitis B and D, and to develop preventive measures aimed at minimizing the prevalence of these diseases in the country.

Methods & Materials: A descriptive study was conducted from 1st January-31st November 2014 at RAHILA Research & Reference laboratory, Karachi, Pakistan. A total number of 500 serum samples were collected from patients coming to multiple clinics for diagnostic HDV testing at various public sector hospitals in Southern Punjab & Sindh, Pakistan.

Results: The results showed that 105/500 (21%) tested positive for HDV, 5/500 (1%) tested border line while 390/500 patients (78%) were negative for HDV by ELISA method.

Conclusion: This study aimed to provide epidemiological burden of HDV in Pakistani population. The results show that a moderate percentage of study cohort was tested positive indicating the significance of HDV testing alongwith HBV. HDV testing can serve as management tools for clinicians for therapeutic purpose.
Outbreak of measles in Bhelterghat, Ghanapara and Pekbeki, Goalpara, Assam, India, 2015
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**Background:** Among 21.5 million children globally who did not receive a single dose of measles vaccine in 2013, 6.4 million (30%) were in India, resulting in a large susceptible population at risk for propagating measles outbreaks. On March 20, 2015, a single health facility in Goalpara District in Assam reported 73 children < 18 years with fever and rash. We investigated the suspected measles outbreak to confirm and describe the outbreak, estimate vaccine effectiveness, and propose recommendations for prevention and control measures.

**Methods & Materials:** We defined a case of measles as fever and maculo-papular non-vesicular rash with cough, coryza, or conjunctivitis in three villages (Bhelterghat, Ghanapara and Pekbeki) from 31 January 2015 to 12 April 2015. We conducted a retrospective cohort study of children 6 months to 18 years in the three villages. We interviewed mothers of children and reviewed vaccination cards and immunization registers at health care facilities to assess vaccination coverage and vaccine effectiveness. We tested five serum samples for IgM antibodies for measles towards the end of the outbreak.

**Results:** We identified 103 cases with an attack rate of 8%. There were no hospitalizations and no deaths. Attack rate was highest in children aged between 6-9 months (92%) followed by 10-24 months (19%). Among 103 cases, 53% were female. Most cases (65%) lived in Bhelterghat (10% attack rate) followed by 21% in Ghanapara (3% attack rate) and 14% in Pekbeki (2% attack rate). All cases were treated with vitamin A supplementation during the outbreak. Among the cases, 20 (19%) were vaccinated against measles. Among children 6 months to 18 years, vaccination coverage for first dose measles-containing vaccine was 37% (500/1359) and Vaccine effectiveness of measles-containing vaccine was 60% (95% CI= 33.4 – 74.3). All five serum samples were positive for IgM antibodies for measles.

**Conclusion:** This measles outbreak was caused by low vaccination coverage in the area and subpar vaccine effectiveness. We recommended annual trainings every year for all staff involved in vaccine service delivery and vaccine management. We also recommend strengthening routine immunization in the area by increasing community awareness about vaccines and by increasing outreach vaccination services.
Background: The human papillomavirus (HPV) is the main cause of cervical cancer in developing countries. HPV is common in all socioeconomic groups and is spread all over the world. There are more than 100 types of HPV which are divided into high risk and low risk groups. Molecular method is one of the rapid and accurate methods to study HPV genotypes.

Methods & Materials: The study was conducted in 417 married women with Pap smear samples, referring to gynecology clinics in health center of Firoozgar hospital. The detection of 22 HPV genotypes was performed by using the Multiplex PCR technique. HPV DNA was extracted from positive samples using EzHigh TM DNA Extraction kit. The epidemiological survey of HPV positive samples were evaluated using SPSS 16 software.

Results: HPV was detected in 159 of 417 (38.1%) married women aged 17-85 years. In 22 different age groups among women infected with human papillomavirus, HPV16 (19.1%) was the most frequent HPV types, followed by HPV 39, 18 (12.5%). The highest rate of HPV infections was observed at the age 36 (7.7%). HPV 16 is most common high risk types among HPV positive women who had malignancy history but HPV 35 and HPV 38 are more frequent in women with underlying diseases.

Conclusion: To determine genotypes of HPV and disease management, accurate screening programs are required. Study of prevalence of HPV in each geographical region could be essential to design effective strategies for vaccination against HPV.
Prevalence of urinary tract infection among HIV patients in Aba, Nigeria

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Background: People living with Human Immunodeficiency Virus (HIV) are more prone to opportunistic infection including urinary tract infection (UTI) due to progressive immune dysfunction by the virus. The study was conducted to determine the prevalence of UTI among HIV patients attending Seventh Day Adventist Hospital Aba (SDA) for routine antiretroviral therapy as well as the effect of CD4+ count on the prevalence of UTI.

Methods & Materials: Clean catch mid stream urine and blood was collected from 104 HIV positive patients between June 2015 – September 2015 comprising of 35 men and 69 women. The HIV patients consisted of 67 highly active antiretroviral therapy (HAART) subjects and 37 HAART naïve subjects. Urine samples were cultured, isolates identified and antibiogram was carried out using standard microbiological procedures. CD4+ T-cell counts were measured by the FACS count system using the FACS flow cytometer.

Results: The overall prevalence of UTI was 40.39% with 52.17% occurring in females and 17.14% in males which was statistically significant. Escherichia coli (68.18%) was the most predominant isolated pathogen followed by Klebsiella pneumonia (15.91%), Enterobacter spp (11.36%) and Staphylococcus aureus (4.55%). In the relationship between UTI and CD4+ count a greater percentage of UTI infected participants had CD4+ count greater than 200 cell/µl (41.33%) than those with CD4+ < 200 cell/µl (37.93%) which was statistically non significant. Age group 60 – above had the highest prevalence of 100% followed by age group 30 – 44 (44.9%) which was statistically non significant cell/µl. Only HIV patients on HAART had non significantly higher prevalence of UTI compared with non HAART users (41.79% vs 37.84%) (P > 0.05). The antibiogram showed that the drugs of choice were gentamycin (93.18%) and ciprofloxacin (72.73%). Most bacterial isolates were resistant to nalidixic acid (84.09%), trimethoprim – sulfamethoxazole (63.63%) and Ampicillin (56.09%).

Conclusion: This study draws attention to high prevalence of UTI among HIV patients in Aba metropolitan. Therefore, there is need for HIV patients to be examined for UTI.
Outbreak investigation of acute viral hepatitis in Kangra valley, Himachal Pradesh, India, 2014-2015

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**Background:** India is endemic for viral hepatitis A (HAV) infection with 66% of states reporting at least one outbreak from 2011 to 2013. Following media reports in January 2015, we investigated a jaundice outbreak in Kangra town of Himachal Pradesh Valley with the following objectives: (1) assess the magnitude, (2) identify the source, and (3) initiate preventive measures.

**Methods & Materials:** An active case search was done in Kangra to identify case-patients who had acute onset of jaundice during 1st November 2014 to 23rd January 2015. We conducted a 1:1 case-control study; data on exposures were collected using a structured questionnaire. Serum specimens from five cases were tested for IgM anti-HAV and IgM anti-hepatitis E virus. End-point water specimens from households of case-patients were tested for faecal coliforms using most probable number method.

**Results:** The overall attack rate was 1.6 (149/9528). There were no deaths. Illness began on November 3rd, 2014 and persisted through January 21st, 2015. Among 149 patients, 62% were in 5-14 age-group with no gender differences. All five serum samples tested positive only for IgM anti-HAV; no contamination was detected in water samples. Contact with jaundice patients in previous two weeks (OR 1.6; 95% CI 1.03-2.6; p<0.01), drawing water by dipping ladle in storage containers (3.2; 95% CI 2-5.2; p<0.001), storing drinking water in narrow necked containers (16.9; 95% CI 7.7-36.9; p<0.001), past history of jaundice (OR 15.3; 95% CI 1.9-118; p<0.001) and not washing hands with soap before meals (OR 3.8; 95% CI 2.0-7.1; p<0.001) were associated with illness.

**Conclusion:** The findings suggested that this was an outbreak of hepatitis A. Promotion of personal hygiene and sanitation among residents of Kangra was recommended.
Prevalence of severe rotavirus associated gastroenteritis among children under five years of age in Chennai, India
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Background: Rotavirus is the major cause of severe gastroenteritis affecting infants and young children globally. The Indian Council of Medical Research funded National Rotavirus Surveillance Network carries out hospital based surveillance to estimate the prevalence of rotavirus among children under 5 years of age hospitalized with gastroenteritis in different parts of India, adding new facilities as needed for surveillance. In this report, we present findings from the first year of surveillance carried out in the largest pediatric care facility in Chennai, a metropolis in southern India.

Methods & Materials: Children under five years of age admitted with acute gastroenteritis were screened and eligible children were enrolled. A standardized clinical recruitment form was used to collect demographic and clinical details and treatment outcome. Stool specimens were screened for Group A Rotavirus by ELISA and G and P genotyping was carried out on a subset of positive samples using hemi-nested reverse transcription PCR.

Results: During the 13-month period between July 2014 and August 2015, 759 eligible children were enrolled. Stool specimens from 670 children were tested and group A rotavirus was detected in 32.1%. Age-wise analysis showed that the highest positivity (39%) was seen among children aged 12-23 months followed by children aged 6-11 months (36.5%). Most rotavirus infections occurred during December to February (48.9%). Among rotavirus infected children, 87% had vomiting and 77.2% had ≥ 6 diarrheal episodes in a 24h period. Diarrhea disease severity analysis using Vesikari score revealed that 65.6% of rotavirus infected children had severe to very severe disease. G1P[8] (67.6%) was the most common rotavirus genotype followed by G9P[4] (19.1%).

Conclusion: Surveillance data documents the high burden of severe rotavirus associated gastroenteritis among young children in Chennai and underscores the importance of rotavirus vaccination in tackling this major public health problem.
The effect of hospital visit behavior on the outcome of severe hand, foot and mouth disease in middle China
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Background: Epidemics of hand, foot and mouth disease (HFMD) are elevated every year globally, especially in mainland China. And there is no safe and effective vaccine or antiviral medications available worldwide at present. The disease now deeply affects children’s health and presents as an increasing threat to public health.

Methods & Materials: A case control study was designed to examine the effect of hospital visit behavior on the outcome of severe hand, foot and mouth disease from January 2011 to December 2014 in Hunan, China. A total of 576 HFMD cases (276 death cases as the case group and 300 survival cases as the control group) were collected from the National Surveillance System. Multi-factorial logistic regression was used to analyze independent associations between hospital visit behavior and death from severe HFMD.

Results: The male-to-female ratio was 1.51 : 1 in the death cases and 1.78 : 1 in the survival cases. Over 90.0% of cases are children under 3 years of age. 214 (77.5%) and 98 (32.7%) of death and survival cases first visited a village level clinic (OR = 9.548, 95% CI: 3.22-35.37). Rates of confirmed diagnosis at first visit to hospital among death and survival cases were 22.8% and 68.6% (OR = 0.44, 95% CI: 0.17-0.89). 67 (24.3%) of death cases died in village level hospital. Only 6 (2.0%) of survival cases discharged from village level hospital (OR = 11.92, 95% CI: 4.70-30.25). 201 (72.9%) and 187 (62.4%) of death and survival cases consulted the doctor within three days of symptoms developing (OR = 2.43, 95% CI: 1.06-5.59). 13.5% of death cases didn’t have any medical insurance, the rate among survival cases was only 1.7% (OR = 8.36, 95% CI: 2.98-23.46).

Conclusion: Changing some hospital visit behavior of patients may help reduce the mortality from severe HFMD. And health administrative departments should focus on rational allocation of health resources, optimization of health service systems and increase input into village level health institutions.
Community cases management of malaria in Tripura, India- MSF intervention in response to malaria epidemic

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\textbf{Background:} The international medical humanitarian organisation Médecins Sans Frontières (MSF) launched a medical intervention to respond to the unprecedented malaria epidemic in Tripura state, India between July and September 2014. The objective was to reduce morbidity and mortality due to malaria outbreak in Tripura through a community approach of improved access to a comprehensive package of malaria prevention, diagnosis and treatment in the most affected villages of Dhalai district.

\textbf{Methods & Materials:} This was an observational study depicting MSF response to malaria epidemic in Dhalai district. Following an assessment in July 2014, MSF has started its intervention. Two malaria clinics were set up by MSF in Longtharai Valley. These centres ensured early diagnosis using Malaria rapid diagnostic tests and treatment as per Artemeter and Lumefantrine (ACT-AL) based protocol. The community approach to test and treat uncomplicated malaria at village level was implemented through trained community health workers (CHW) in seven outreach sub-bases. These CHWs were also trained to identify symptoms of severe malaria which were then being referred to the nearest health facilities. Health Promotion activities were carried out by means of health education and distribution of Long Lasting Insecticidal Nets (LLIN).

\textbf{Results:} During intervention period of 3 months a total of 5267 malaria rapid tests were performed among suspected cases. A total of 2327 (44.2\%) cases were diagnosed positive. Of these 616 (26.5\%) were children less than five year of age. Among positive cases 52\%, 43\% and 5\% cases were diagnosed with Plasmodium falciparum, Mixed and Plasmodium vivax respectively. All diagnosed patients were treated on the spot. Nine severe cases were referred to nearest health facility. A total of 2550 LLIN were also distributed.

\textbf{Conclusion:} We believe that community approach has proven to be an effective mean for management of malaria outbreak in poor resource setting. We conclude that CHW are able to provide good quality malaria care, including screening cases using case definition and perform rapid diagnostic tests. Appropriate training, clear guidelines, and regular supportive supervision are important facilitating factors. Crucial to success of CHW programs is strengthening health system capacity to manage commodity supply, disease surveillance, supervision, and appropriate treatment of referred cases.
Morbidity by Influenza A (Novel H1N1) virus infection in relation to age and gender from January 01, 2015 to October 30, 2015 in Baroda, a city in western India

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Background: Aim of this study to identify potential high risk age group and gender predisposition for the Influenza A (Novel H1N1) virus infection.

Methods & Materials: Retrospective analysis of data from urban and rural population of Baroda has been carried out by principal investigator. The statistics were collected over a span of approximately 10 months, from January 01, 2015 to October 30, 2015. Two swabs, Oropharyngeal and nasopharyngeal, were collected by Nylon flocked swab and following WHO recommended standardized protocols. Swabs were transferred to Viral Transport Media (VTM). Triple layer packing was done using Biohazard Zip lock bag and cotton as absorbent and All the samples were transported in cold chain to referral viral laboratory for testing. Further virus detection was done via real time reverse transcriptase polymerize chain reaction machine, the gold standard method.

Results: But of 1,650 patients, 657 patients tested positive for Influenza A (Novel H1N1) virus infection, with the positivity rate of 39.82%. Among them, 326 (49.62%) were males and 331 (50.38%) were females. Majority of cases occurred during the month of February and March. In February, 418 (50.00%) patients tested positive out of 836 patients, with 203 (48.56%) males and 215 (51.44%) females. In March, 146 (30.04%) patients tested positive out of 486 patients, with 74 (50.68%) males and 72 (49.32%) females. Out of 657 patients, 120 (18.26%) patients were from pre-school (0-5 years) age group, highest among the all age groups. Significant number of cases also occur in middle age groups, 64 (09.74%) in 51-55 years, 52 (07.91%) in 36-40 years and 50 (07.61%) in 46-50 years.

Conclusion: Study saws both genders are affected with equal rate with no predisposition to either male or female. Pre-school (0-5 years) age group has high risk of infection and middle-age groups (36-55 years) have moderate risk as compared to other age groups. Results of this study emphasizes the need for further research and evaluation as well as comprehensive surveillance system to know more about Influenza A (Novel H1N1) virus infection in relation to age and gender.
Estimation of the chronic obstructive pulmonary disease from exposure to particulate matter in Ahvaz, Southwest Iran

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Background: Air pollutants produced in environments have many harmful effects on human health. Chronic obstructive pulmonary disease (COPD) is a common worldwide respiratory disease. The aim of this study was to evaluate the relationship between the density of particulate matters and the prevalence of COPD in Ahvaz, southwest of Iran, during 2009-2013.

Methods & Materials: This epidemiological and used-model study was performed in Ahvaz. Data were obtained from Ahvaz Department of Environment (ADE). Sampling was performed hourly during the study period in 4 stations. In this study, 175200 samples of air were taken and collected. Sampling and analysis were performed according to EPA guideline. We utilized the relative risk values and baseline incidence measures by the WHO (Middle East) drawn from Health Effects Association of Particulate Matter. Finally, prevalence of COPD attributed to particulate matter exposure was calculated by Air Q model.

Results: According to our findings, the prevalence of COPD attributed to particulate matters increased during 2009-2013 and followed an increasing trend. Accordingly, the yearly prevalence of COPD during the period 2009-2013 were 1026, 981, 1235, 1602, and 2625,, and the yearly average PM_{10} concentrations during the same period were 277.64, 261, 323.78, 727.65, and 917.12 µg/m³, respectively. The total mean of particle matter concentration was higher than standard. There was a strong correlation between the prevalence of COPD and the concentration of particulate matters in Ahvaz.

Conclusion: Our study suggested that increased concentration of particulate matters might be associated with a higher prevalence of COPD.
First molecular detection and genotyping of group A rotaviruses by semi-nested RT-PCR from Sewage in Nigeria

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Background: Rotavirus is the leading cause of viral gastroenteritis worldwide; sewage and sewage polluted waters have long been identified as a major source of rotavirus dissemination in the environment. The objective of this study was to detect and identify the circulating G genotypes of rotavirus in sewage from Nigeria using molecular method.

Methods & Materials: From June 2014 to January 2015, 190 sewage samples were collected from 5 states in Nigeria, Borno, Sokoto, Abuja, Kano and Lagos. The two phase concentration method using PEG 6000 and dextran was used to concentrate sewage samples following WHO protocols. Molecular detection was done by RT-PCR, semi-nested multiplex PCR was used to genotype the G protein coding region (VP7) and P protein (VP4).

Results: Results showed that 14.2% (n=27) of the samples tested positive for rotavirus RNA. Monthly distribution showed that the rainy months of June to September had a lower detection rate of between 3.7% to 7.4% compared to the dry months of October to January 11% to 26%. Genotype distribution showed a higher diversity of G genotypes than P, G1 predominated followed by G8, G9, G4 and lastly G2, only 2 P genotypes were encountered [P4] (60%) and [P8] (20%), G1[P4] was the most prevalent genotype combination, about 22.2% (n=6) of isolates were untypeable by the primers used.

Conclusion: Our results report the detection and G genotype distribution of rotavirus from sewage in Nigeria for the first time. Genotype G1[P4] was the most prevalent genotype with a high level of untypeable rotavirus strains, this call institution of molecular surveillance incorporating environmental and clinical surveillance in light of introduction of rotavirus vaccination in Nigeria.
Evaluation of risk factors that have the potential for the introduction and spread highly pathogenic avian influenza and Newcastle disease into two states of Nigeria

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\textbf{Background:} The risk of virus introduction and spread into or within farms depends largely on neighbouring farm characteristics, contact structure and biosecurity practices. A qualitative risk analysis was conducted through a cross-sectional study to obtain information on types and frequency on activities guiding the determination of potential AI spread pathways between farms and production regions.

\textbf{Methods & Materials:} The study involved a 95\% responded structured questionnaires that were complemented by dialogues administered to poultry farmers, traders and experts (Veterinarians and livestock health workers) in two Nigerian States.

\textbf{Results:} 92\% of farmers were aware of and 90\% of them were prepared to report outbreaks of poultry diseases. Farmers believed strongly (90\%) that contaminated feed and water caused most poultry disease outbreaks. Of concern is no farmer (0\%) believed ND could be transmitted by wild birds. Veterinary personnel and radio and television contributed the most (87\%) to HPAI awareness. Gombe state had 3 moderate rate reported (12 scores) sites involving trade in live birds and returning trucks from infected areas while 1 high risk (20 scores) area involving trade in live birds. Bauchi state recorded 5 moderate areas (12-13 scores) involving returning trucks, contaminated eggs from infected areas and wild birds while a high risk (20 scores) was established. Chisquare ($\chi^2$) analysis showed that LBMs had significant association ($P = 0.004$) with the prevalence of AI but no such association ($P = 0.147$) with ND. Temperature ($P = 0.033$) rainfall ($P = 0.033$) were important meteorological factors associated with seropositivity of AI while altitude ($P = 0.001$), humidity ($P = 0.000$) and rainfall ($P = 0.003$) were strongly associated with ND in this study. Further, the odds ratio at 95\% CI (1.313-6.333) showed three times likelihood of AI and ND occurrence in the presence of LBMs.

\textbf{Conclusion:} There existed moderate to high risk of AI introduction and spread in the two states. Newcastle disease was a major threat to poultry farming, other endemic poultry diseases contributed significantly to economic losses in these States. Risk assessment and participatory disease investigation would give an early warning to inform strategic livestock policy reforms in Nigeria and other developing nations.
What are the burden and spectrum of skin infections in Cameroonian prisons?


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Background: Sub-Saharan African (SSA) prisons are characterized by overpopulation, promiscuity, poor hygiene, precarious quality of life and total lack of health care, which are known drivers of skin infections (SI). The present study sought to investigate the burden and spectrum of SI among detainees in Cameroon, a SSA country.

Methods & Materials: Three prisons were randomly selected in the West region of Cameroon where we consecutively recruited 755 inmates who voluntarily accepted to participate in the study. They were independently examined by two well-trained and experienced dermatologists, the diagnosis being confirmed on the basis of clinical findings. Discordant cases were resolved by discussion and consensus.

Results: Ages of participants ranged from 14 to 82 years with a mean of 32 ± 12 years. There were 17 females (2.3%). We recorded a total of 324 SI (42.9%; 95% confidence interval (CI): 39.4-46.4%). They were dominated by parasitic infections: human scabies (242/324: 74.7%) and bodily pediculosis (1/324: 0.3%); then followed fungal infections: dermatophytes (33/324: 10.2%), pityriasis versicolor (29/324: 9.0%), onychomycosis (14/324: 4.3%), and microsporic ringworm (1/324: 0.3%). Lastly, bacterial infections were recorded: impetigo (2/324: 0.6%) and furonculosis (2/324: 0.6%). Age (p = 0.918), sex (p = 0.919), marital status (p = 0.358) and religion (p = 0.505) were not associated with the occurrence of SI. Contrariwise, after multivariate logistic regression analysis, low level of education (adjusted odds ratio 1.54, 95% CI: 1.13 – 2.10; p = 0.006) remained an independent factor increasing the likelihood to be affected. Of note, other dermatoses were encountered: eczema (49 cases), prurigo (5 cases), urticaria (3 cases), seborrheic dermatitis (2 cases), acne (34 cases), keloids (5 cases), and ichthyosiform xeroderma (2 cases).

Conclusion: Skin infections are preponderant in Cameroonian prisons, mainly dominated by human scabies and dermatophytes and perhaps highlighting the poor health care delivered in these milieus. Urgent measures need to be taken to enhance and strengthen the health care provided in our penitentiaries. In this regard, the health personnel working there should be trained to properly diagnose and manage these pathologies, and medicines made available free of charge.
Assessing the baseline burden of otitis media in children 2 to 3 years of age for estimating the effects of 13-valent pneumococcal conjugate vaccine (PCV) on otitis media

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Background: Otitis media is a common illness resulting in major resource use in children under-five years of age in high income countries. However, there is paucity of data on its epidemiology and clinical presentation in low-resource settings. We aimed to estimate the disease burden of otitis media in Cameroon and to determine the possible risk factors.

Methods & Materials: A population-based cross-sectional descriptive prevalence study of otitis media (OM) was performed on randomly selected children aged 2 to 3 years in Yaounde, Cameroon. Two rounds of studies were planned: first for PCV-unvaccinated children between March and June in 2013, and the second round for PCV-vaccinated children 2 years later. OM burden was estimated using tympanometry and pneumatic otoscopy for otitis media with effusion (OME) and by parental questionnaires for acute otitis media (AOM). Here we report the baseline results from the first round.

Results: Out of the 529 children enrolled, 427 were included in the final analyses and the rest were excluded due to indeterminate tympanogram data. Nonetheless, there were no major differences in the baseline characteristics between the two groups. Of those subjects, 84/427 (19.7%) were diagnosed with at least one form of otitis media or its complications. This consisted of 21 (4.9%) children with bilateral OME (i.e. OM in both ears) and 44 (10.3%) with unilateral OME. Based on otoscopy and parental questionnaire, an additional 14 (3.0%) children were diagnosed with AOM, 3 (0.7%) with unilateral CSOM based on inspection and 2 (0.5%) with unilateral ear perforations. No statistically significant relationship was found between OME and any of the predictor variables. However, multivariate logistic regression analysis identified a strong association between the existence of OME and one health outcome i.e. notification of any ear related problems/symptoms in the last 6 months prior to study period (Odds Ratio: OR = 2.5 and Confidence Interval: 95% CI = 1.3 – 5.0).

Conclusion: The findings indicate that as many as every one in five children in our study population were affected by middle ear disease between the ages of 2 and 3 years.
Staphylococcal foodborne illness outbreak, Tshwane District, Gauteng Province - South Africa, June 2015

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Background: Staphylococcus enterotoxin A is one of the few Staphylococcus aureus strains producing gastroenteritis symptoms and is a common cause of food-borne illness worldwide. Although food-borne illness is a notifiable disease in South Africa, its burden is not well known, possibly due to under-reporting. On May 31 2015, three public hospitals in Pretoria notified Tshwane-Outbreak-Response-Unit (ORU) of 51 cases presenting with vomiting and stomach cramps following consumption of food at a local hotel on May 30 2015. All patients were treated and discharged within 24 hours. The ORU conducted further investigations to determine the magnitude, clinical-manifestation and likely source of the outbreak.

Methods & Materials: On June 1st 2015 the team visited the three affected hospitals and collected demographic and clinical details of those affected. We interviewed potentially exposed persons using a structured questionnaire. Food samples (chicken, cabbage, rice, brown onion soup) were collected from leftover food and sent to the laboratory for enterotoxin testing. Clinical specimens were not collected.

Results: Of the 50 potentially exposed persons, 37 were cases. The reported symptoms included: abdominal cramps (37/37, 100%); vomiting (32/37, 86%); nausea (13/37, 35% fever (9/37, 24%); diarrhea (12/37, 43%). The mean age was 23 years (range 9 – 58 years) and males were the most affected group (76%, 28/37). The median incubation period was 2.5 hours and symptoms lasted a median of 24 hours. The food-specific attack rate was 74% (37/50) among those exposed to chicken and 28% (14/50) among those who ate rice and/or brown onion soup. Staphylococcus enterotoxin A was isolated from the chicken and no pathogens were isolated from other foods.

Conclusion: Staphylococcus enterotoxin A was the likely cause of the outbreak with poor hand hygiene practices among food handlers being the likely source of infection. The relationship between staphylococcal foodborne illness and poor hand hygiene among food-handlers is well established in the scientific literature with cross contamination frequently occurring during food preparation. Ongoing hand washing awareness should be conducted to improve hand hygiene practices among food-handlers in affected food establishments. Training could improve the knowledge and outbreak response capacity among the environmental health practitioners and emergency unit health care workers.
Factors associated with high HIV related stigma among commuter populations in Johannesburg inner city, South Africa

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Background: Stigma remains the single most important barrier to individual confidence. Despite education about stigma and its morphing characteristics, it has continued to exist. The main objective of this study was to identify factors related to HIV stigma among a commuter population in the Johannesburg inner city of South Africa.

Methods & Materials: Data were collected using a self-administered closed ended questionnaire loaded onto personal tablet computers during a community outreach campaign. All measures were self-reported. The outcome was measured by asking the respondents to rate their perceptions of levels of stigma as ‘high or low’.

Results: A total of 1146 participants were involved in the study of which 585 (51.0%) reported high–levels of stigma. Overall, being married/cohabiting (aOR: 1.47 95%CI: 1.05-2.04), divorced (aOR: 4.36 95%CI: 1.48-12.81), aware of HCT services (aOR: 2.10 95%CI: 1.40-3.14) and a preference for home/mobile outreach HIV testing centres (aOR: 1.40 95%CI: 1.03-1.89) were associated with high levels of stigma. Female participants who preferred HIV testing at home or from a mobile outreach centre (aOR: 1.60 95%CI: 1.06-2.45) were more likely to report high levels of stigma. However participants who were employed (aOR: 0.50 95%CI: 0.37-0.68), perceived HIV risk as high (aOR: 0.10 95%CI: 0.07-0.14), and knew HIV testing benefits (aOR: 0.45 95%CI: 0.33-0.61) were less likely to report high level of HIV-related stigma.

Conclusion: High level of stigma to HIV infection still exists in our communities. Therefore enhancement of health education and reinforcing the benefits of knowing personal HIV status is essential to overcome stigma, a known obstacle for expanding access to HIV testing and counselling.
Development of saliva based diagnostic method for malaria

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Background: More than half of the world’s population are exposed to malaria. Rapid diagnosis and proper treatment are the main aim of control programs in malaria endemic areas. This study was aimed at developing a non-invasive diagnostic method for detection of *Plasmodium falciparum* in human malaria.

Methods & Materials: Ethical permit was obtained from CUREC. Informed consent/assent was also sought. Saliva and blood samples were collected from patients presenting with fever at the Covenant University Health Centre. Falciparum malaria was confirmed by microscopic examination of Giemsa stained thin and thick blood smears. Direct nested PCR was carried out to detect drug resistant genes (pfcrt and pfmdr) from positive blood and corresponding saliva samples using TransDirect Animal Tissue Kit (Transgene Biotech, Beijing).

Results: Out of the 165 subjects recruited on this study, 61.8% was positive for falciparum malaria by microscopy. The presence of pfcrt gene was detectable from 20 blood samples while only 10 blood and saliva samples from same patients were harbouring the pfcrt gene.

Conclusion: The use of human saliva as sample for malaria detection would help facilitate effective diagnosis and development of simple probes which will be easier to handle especially in malaria endemic areas where proper diagnosis is still lacking.
Outbreak investigation of Kaysanur Forest Disease (KFD) in Wayanad district, Kerala, India 2015

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Background: Kyasanur Forest disease (KFD), a tick-borne viral hemorrhagic fever has a case fatality rate as high as 10%. KFD is endemic to Karnataka but is spreading. Infected ticks transmit infection to monkeys, the amplifying host. Vaccination of high risk populations is the primary control strategy. On February 6, 2015, an outbreak of KFD was reported in Wayanad district, Kerala near Chikenji forest. We investigated to describe the outbreak and identify risk factors.

Methods & Materials: We defined cases as residents of Sulthan Bathery taluk who during 25 December 2014 - 13 March 2015 presented with fever, headache, and myalgia. House to house survey and health facility-based surveillance was established for case finding. For each case we selected two healthy controls matched for age, sex and place of residence and interviewed regarding exposures. We conducted entomological and monkey death investigations to better understand transmission.

Results: Among 113 cases, including 6 deaths, 62% were females; median age was 40 (3-70). None had received vaccination. Of 81 cases tested by reverse transcription polymerase chain reaction, 43 (53%) were confirmed for KFD virus. Among 59 cases and 118 controls, a recent visit to forest (aOR=4.8, 95%CI=2.1-10.6), grazing animals in forest (aOR=3.1, 95%CI=2.2-6.9), exposure to monkey death (aOR=4.1, 95%CI=2.2-7.2) and collecting heaps of leaves around house (aOR=1.9, 95%CI=1.09-2.8) were significantly associated with illness. The tick vector (Hemophysalis spinigera) for KFD was abundantly found and 18 monkey deaths (5 tested positive for KFD) were reported from affected area.

Conclusion: This was the first reported outbreak of KFD in Kerala with virus, and vector also found in the area. We recommended vector control, use of personal protective measures and vaccination policy for prevention of KFD outbreaks in future.
Molecular epidemiology of rabies virus in Nepal

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Background: Rabies is endemic and priority zoonotic disease in Nepal. Death of 150 people and 200 animals has been reported annually in this country however molecular epidemiology of rabies has not been well understood. A study was performed by analyzing 10 rabid animal's brain samples (7 dogs, 1 goat, 1 buffalo and 1 alpaca) collected in Nepal from 2011-2012 to know the circulating clade of Rabies virus in Nepal.

Methods & Materials: All 10 samples were analyzed in Changchun Veterinary Research Institute, OIE reference laboratory in China in 2012, by performing molecular characterization and phylogenetic analysis. First of all, Rabies virus or antigen was detected in 10 samples by Fluorescent Antibody Test and Mouse Inoculation Test. Viral RNA was extracted from only 8 positive samples by using QIAGEN™ RNeasy Kit. Amplification of N and G genes of rabies viral RNA was performed by using primers with Invitrogen Super Script III First-Strand Synthesis System and the Platinum Taq DNA Polymerase High Fidelity kit (Invitrogen, USA). PCR products were analyzed by Agarose gel electrophoresis (Biorad molecular biology grade agarose). Analysis of sequence was made by using Clustal W of MEGA 5.1 and DNAStar v. 7.2 (DNAStar Inc., USA). Alignment analysis was performed with reference sequences from Nepal and surrounding countries, such as, China, India, Pakistan and Sri Lanka.

Results: All the data demonstrate that viruses isolated in Nepal belong to the classic rabies virus. N gene phylogenetic analysis of Nepalese sequences with reference sequences showed that Arctic related subclades AL-1 and AL-3 are circulating in Nepal. One buffalo brain was AL-1, and 7 dogs brain were AL-3. Further analysis indicates that these Nepalese isolates were genetically close to viruses isolated in India.

Conclusion: The identification of isolates in this study has contributed to our understanding of the natural circulation and transmission of rabies viruses between Nepal and India. However, Himalayan Mountain provides a natural barrier and probably constrains the spread of rabies between Nepal and China. Our findings suggest that national geographical boundaries and border controls act as effective barriers to halt the spread of rabies from Nepal into adjacent regions.
Typhoid fever surveillance in Africa program (TSAP): Constructing a geospatial sampling frame for random sampling of households

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**Background:** Household sampling is used for cost-efficient, in-depth population-based investigations, including the assessment of healthcare utilization. Probability sampling requires a comprehensive sampling frame (household list), which did not exist at sites of the Typhoid Fever Surveillance in Africa Program (TSAP) in Pikine/Senegal, Pietermaritzburg/South Africa, and Wad Medani/Sudan. Here we describe the methodology of constructing a geospatial sampling frame.

**Methods & Materials:** Sites were diverse in population size, population density, area size, and administratively-identified subareas. They varied in topography, vegetation, and composition of formal/informal settlements and single-/multi-story structures. We used high-resolution GoogleEarthPro satellite imageries for manually enumerating (pinpointing) structures to construct a sampling frame at each site. Hand-held satellite maps and global positioning system (GPS) devices were utilized by interviewers for rapidly, precisely locating structures (households); structures were selected randomly and weighted proportional to the population size of each subarea (weighted-stratified sampling). The methodology was evaluated for accuracy by calculating ranges and quartiles of distances between GPS coordinates of original (as per sampling frame) and enrolled structures.

**Results:** A total of 46510, 63008, and 32794 structures were enumerated at sites in Senegal, South Africa, and Sudan, respectively. The fabrication of satellite maps, including the selection and pictorialization of structures, took approximately two to three weeks by site. We found that 87% (517/592), 56% (1,098/1,963) and 86% (471/549) of distances were <2.5 meters at the site in Senegal, South Africa, and Sudan, respectively. Calculations revealed furthermore that 59% of distances in rural, 57% in semi-urban and 50% in urban subareas of South Africa were <2.5 meters.

**Conclusion:** We have demonstrated that satellite imageries are a simple, precise instrument for creating a sampling frame at certain TSAP-sites. Hand-held satellite maps and GPS devices allowed for the rapid, accurate location of selected structures. Some limitations remain. Correctly locating structures can be challenging for clustered, interlaced structures since interfering factors (obstructive/reflective structures, multi-story buildings, environmental diversity) may impact on the precision of GPS readings. Geospatial frames require constant updating, but could provide an approach for population-based investigations at probability samples of households in settings that are uncensused and lack longitudinal recording of socio-demographic and vital statistics.
Genetic variability of the G-L intergenic region sequences of Indian rabies virus strains circulating in animals

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Background: The evolutionary studies of rabies virus (RABV) have targeted the N or G gene, or recently the G-L intergenic region sequences. Among these, G-L intergenic region is considered as the most variable since it is not subjected to immunological selective pressure. The present study was undertaken to understand the genetic variability of RABV in animals in India based on the G-L intergenic region.

Methods & Materials: Twenty seven brain samples from suspected rabid animals (22 dogs and five cattle) resourced from Karnataka (n=9), Kerala (n=5), Rajasthan (n=3), Tamil Nadu (n=2), Manipur (n=4) and Uttar Pradesh, Gujarat, Puducherry and Jammu Kashmir (n=1 each) were confirmed by Direct Fluorescent Antibody (DFA) assay (Fig.1). The samples were further subjected to Reverse Transcription Polymerase Chain Reaction (RT-PCR) along with Dr. Larghi’s strain (PV-3462) as reference to amplify the G-L intergenic region. The PCR products (1354 bp) were purified, sequenced and compared to the corresponding sequences of RABV from different countries, CVS and PV strains obtained from GenBank. Phylogenetic tree was constructed using the nucleotide sequences corresponding to 423 bp of the 1354 bp amplicon. The branching pattern of the trees was constructed by the Neighbor Joining method using Mega 5 software version 5.02.

Results: The phylogenetic analysis revealed two major groups of RABV in India (Fig.2 & 3): Group 1 circulating all over India and Group 2 restricted to two Southern states, Tamil Nadu and Kerala. Group 2 RABV showed high homology with the Sri Lankan isolates. Group 1 was further sub-grouped into four, designated as 1a, 1b, 1c and 1d. Group 1a included the majority of isolates from Karnataka and Puducherry, and one from Kerala. Group 1b included RABVs from Rajasthan, Gujarat, Uttar Pradesh and Karnataka, whereas Group 1c included an isolate from Jammu & Kashmir along with isolates from Pakistan. Group 1d included isolates from Manipur and Bangladesh.

Conclusion: Rabies viruses circulating in animals in India belong to Genotype 1, and are genetically diverse. In the present study, the sub grouping of RABVs could be due to major geo-physical barriers such as Himalayan range, Western ghats and major rivers including Ganga and Brahmaputra.
Prevalence of rickettsial infections in acute coronary syndromes in Sri Lanka: A case control study
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Background: Interest in the relationship between infection and atherosclerosis induced coronary heart disease has recently increased. Rickettsiae are a group of obligate intracellular pathogens who invade endothelial cells and cause vasculopathy. In a longitudinal nation wide study conducted in Taiwan, the incidence of acute coronary syndromes (ACS) in patients with scrub typhus was found to be higher than a comparison cohort (3.10 vs 1.92 per 1000 person-years). A 37% increased risk in subsequent development of ACS has been demonstrated compared to general population after adjusting for age, sex and other independent risk factors; hypertension, diabetes, hyperlipidaemia, chronic obstructive pulmonary disease and coronary artery disease. The prominent effect of scrub typhus on subsequent ACS development has appeared within 1 year after infection.

Aims: To assess the prevalence of Rickettsial infections in patients with ACS who live in the Western province, Sri Lanka.

Methods & Materials: Patients admitted with ACS to the Professorial Medical Unit, Colombo North Hospital, Ragama, Sri Lanka from April to December 2011 were studied for the serological prevalence of rickettsial infections and were compared with a matched control group; who had no fever or ACS and admitted during the same period. 2 ml serum samples were obtained at enrolment and 2 weeks after, to assess exposure to rickettsial infections by IFA-IgG antibody titres against Orientia Tsutsugamushi (OT) and Spotted fever group (SFG) rickettsioses. An IgG titre ›1:128 or a rising/declining titre were considered positive for acute rickettsioses. A static titre was considered previous exposure to Rickettsioses.

Results: 46 ACS [males n(23.9%), mean age (SD) 61.1(13.1) y] and 52 controls (males n (50%), mean age(SD) 56.0(13.6) y] were studied. None had evidence of acute rickettsial infection. Sero-prevalence of IgG (OT) was 6.4% and IgG-SFG was 15.2% among ACS patients while that of control group were 3.8% and 11.5% respectively. There was no significant difference in sero-prevalence of OT [OR =0.74 (CI, 0.28-10.93), p=0.66] or SFG [OR=1.376 (CI, 0.43-4.44), p=0.59] in patients with ACS compared to controls.

Conclusion: We observed no significant difference in sero-prevalence of rickettsioses in patients with acute coronary syndromes compared to controls in this study.
Influence of α thalassemia on the protective effect of sickle cell gene on severity of P. falciparum malaria

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**Background:** *P. falciparum* malaria and sickle-cell-anemia are two major public health problems in western Odisha. It has been hypothesized that sickle-cell-gene protects against severe *P. falciparum* malaria. Again, African studies described the negative epistatic interaction for protection against malaria between sickle-cell-gene and α-thalassemia when co-inherited together. This study was undertaken to assess the role of α-thalassemia on the protective effect of sickle-cell-gene on severe *P. falciparum* malaria.

**Methods & Materials:** Adults patients with severe *P. falciparum* malaria were included. Age, sex and ethnic matched control (no malarial infection since 5 years) were taken. Sickle-cell-gene and α-thalassemia was confirmed by ARMS-PCR and GAP-PCR respectively. Clinical and haematological data were analyzed.

**Results:** 396 patients were registered, including 284, 66 and 46 patients with HbAA, HbAS and HbSS respectively. In control (total 391 cases), 301 cases had HbAA and 90 cases had HbAS. In HbAA with severe falciparum malaria, the incidence of α-thalassemia was 36.6% (104/284) in patients compared to 47.2% (142/301) in control ($\chi^2$, 6.25; $p=0.012$). In HbAS, the incidence of α-thalassemia was 81.8% (54/66) in patients compared to 52.2% (47/90) in control ($\chi^2$, 13.3; $p=0.0003$). In HbSS, the incidence of α-thalassemia was 63.0% (29/46). In HbAA, patients with α-thalassemia had increased Hb and RBC levels with lowered MCV and MCH compared to normal α-genotype. In HbAA, the incidence of ARF, Jaundice, cerebral malaria and death were significantly low in patients with α-thalassemia. The number of complications has increased with decreased α-globin gene number in both patients with HbAS and HbSS. In HbAS, patients with α-thalassemia had a greater HbA/HbS ratio compared to patients with normal α-genotype ($p<0.01$).

**Conclusion:** Patients with α-thalassemia had better haematological and clinical parameters compared to normal α-genotype in HbAA. The high incidence of α-thalassemia in patients with HbAS, suggest the negative epistatic interaction of α-thalassemia on the protective effect of HbAS against severe malaria. This hypothesis again supported by high HbA/HbS ratio in our patients with HbAS and α-thalassemia. Longitudinal cohort study is essential to understand the pathophysiology of malaria and haemoglobin disorders in India.
Role of medical colleges in TB control under RNTCP - Five years experience in Puducherry, S. India (2010 - 2014)

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**Background:** A substantial proportion of patients with TB are managed at medical colleges across India. The RNTCP of Govt. of India conceived and implemented the unique experiment over a decade ago of involving the academicians who constitute the faculty in the public health programme for TB control by a mechanism of National, Zonal and State level Task Forces. A periodic review of role of Medical college in TB control is important to monitor the progress.

**Methods & Materials:** Puducherry at U.T. in Southern India with a population of 1.2 million has nine medical colleges, two are government and seven are private institutions involved in implementing the RNTCP and report their progress in a structured format every quarter to the State Task Force (Puducherry) which is reviewed and feedback provided to all concerned. A consolidated report submitted to the Zonal and National Task Forces of RNTCP.

A record based study of the RNTCP STF Quarterly reports from 2010 to 2014 was conducted to find the proportion of TB patients screened, the proportion of sputum smear positive, negative and extra pulmonary TB patients diagnosed, the proportion of patients referred for treatment and proportion of pre-treatment loss to follow up among them.

**Results:** During the study period in Puducherry U.T. a total of 1,14,720 TB suspects were screened for sputum AFB of which 67,174 (58.5%) was in the 9 Medical colleges this ranged from 45.5% to 64.5%. Among those screened, 8,183 (12.2%) were sputum positive TB cases, 3,487 (5.2%) sputum negative TB cases and 2,079 (3.1%) were extra-pulmonary. Thus a total of 13,749 TB diagnosed cases, 398 (2.9%) were initiated on treatment at the Medical College and most referred for treatment as per RNTCP guidelines. It was noted that 11% of sputum positive TB patients had pre-treatment loss to follow.

**Conclusion:** Medical colleges in Pondicherry have an important role under RNTCP in TB case finding, initiation of treatment and referral of TB patients. Strengthening of the referral mechanism is required to prevent the pre-treatment loss to follow up of TB patients.
Evaluation of chikungunya virus infection and screening of antibodies.

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Background: Chikungunya virus infection is now a global health problem, transmitted by aedes mosquitoes. Nearly all patients with CHIKV infection suffer with arthralgia that is usually symmetrical. Fingers, wrists, ankles, elbows, toes, and knees are most often affected. The acute signs and symptoms of CHIKV infection usually resolve within 1–2 weeks, but the arthralgia can persist for months or years. The study done was aimed to evaluate chikungunya infection in 3 different regions of India, during 2009-2010.

Methods & Materials: IgM-ELISA and RT-PCR were used CHIKV detection. Viral load was determined from CHIKV confirmed cases by Real-time PCR. The levels of cytokines (IFN-Y, IL-2) were determined by BD Elisa kit in paired samples. Phylogenitic analysis of the 17 partial E1 gene sequences was done using Mega software. Selected samples were stored in -80°C for further screening, isolation and characterization of chikungunya antibody secreting B-cells. Neutralizing ability will be tested.

Results: Out of 723 tested 249/723 (34.44) were found CHIKV positive. 183/723 (25.3%) by IgM Elisa and 69/412 (16.75%) RT-PCR. Rashes, joint pains and joint swellings were frequently detected in confirmed cases. The qRT-PCR results of 55 confirmed positives showed viral load in the range of 1.66X10⁵ to 9.94 X10⁹ copies/mL. Phylogenetic analysis showed that study strains belong to Central/East African genotype (ECSA). 35 paired samples were analysed for cytokines (IFN-Y, IL-2). Three trends (increasing, decreasing, no trend) were observed for the levels of IFN-Y, IL-2 among acute and convalescent samples. Screening for Chikungunya specific antibody secreting B-cells is under progress.

Conclusion: This study gave us an idea of the proportion of chikungunya infection among suspected people in different regions of India. Comparison of Mean log viral load and cytokine levels with clinical features of CHIKV positive patients showed differences with presence/absence of certain clinical features. Circulating strains belong to Indian Ocean lineage of ECSA strains. Prospective screening of antibody secreting B-cells and isolation of CHIKV specific antibodies will help us in developing interventions for passive immunotherapy. Once the antibodies are ready we plan to test “Shot at site” hypothesis for quick pain relief by giving antibody shots at the site of joint pain.
Clinical, social, and meteorological factors associated with dengue and malaria diagnosis in adults in Pune, India

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Background: Acute febrile illness is one of the most common reasons for hospital admission in India. We sought to assess factors associated with distinct and similar presentation of malaria and dengue that create a diagnostic challenge for clinicians when rapid diagnostics are not available.

Methods & Materials: Patients >12 years of age admitted to adult medicine wards at BJ Medical College - Sassoon General Hospital, in Pune, India with >1 day of fever were enrolled into a prospective cohort between July 2013 and September 2015. Enrollees underwent malaria rapid test (SD bioline) or smear and dengue early ELISA (PanBio), blood cultures and rapid tests for chikungunya and influenza. We assessed demographic, clinical, social, and meteorological data for univariate association with diagnoses of dengue and malaria separately. Factors that demonstrated univariate association were used to construct multivariate models to assess for independent association.

Results: Of 786 adults and adolescents enrolled, 681 underwent dengue and malaria testing. Dengue was positive in 83 (12.2%), malaria in 61 (9.0%), both malaria and dengue in 2 (0.3%) patients. In a multivariate model shown in the figure, elevated humidity 14 days prior to hospital presentation was associated with both dengue (odds ratio (OR) 2.3, 95% confidence interval (CI) 1.7-3.3) and malaria (OR 1.4 (95% CI 1.03-1.94)). Farmers or laborers were more likely to have malaria (OR 2.9 (95% CI 1.5-5.6)) and patients with a child under age 5 at home were less likely to have malaria (OR 0.42 (95% CI 0.2-0.9)). Patients with low income had lower odds of dengue diagnosis OR 0.25 (0.1-0.6) and patients admitted after a higher environmental temperature 2 weeks prior to admission were more likely to be diagnosed with dengue (OR 1.7 (1.2-2.5)). Leukopenia, thrombocytopenia, and elevated hemoglobin were associated with dengue (Fig 1). Thrombocytopenia and low phosphorus were associated with malaria (Fig 2).

Conclusion: Preceding hot and wet weather is associated with dengue and malaria diagnosis among patients admitted with acute febrile illness in Pune, India. Although the finding of thrombocytopenia is present in both patients with dengue and malaria, elevated hemoglobin and leukopenia were associated with dengue and low phosphorus was associated with malaria.
Decadal study of incidence and control of Malaria in Tribal Population – with special reference to Khammam district of Telangana State

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Background: It is stated that 80 percent of the malaria cases in India are confined to 20 percent population of which tribals living in hilly, forest and mountain slopes are significant. Low level of literacy, ignorance and poor health seeking behavior of the tribals are the major reasons for the prevalence of malaria in the agency areas inhabited by the tribals. Health facilities are limited to most of the tribals in view of the logistic problems and inaccessibility. Geographical isolation, remoteness, persistence of vector – conducive environment, poverty, low level of awareness, traditions and superstitious, malnutrition. This research study takes place in Khammam district of the Telangana State where S.T. Population is 27.4 percent, the highest among the districts of South India. The tribal districts of the State are on the edges of the river Godavari. Khammam district is located close to chattisgarh and odisha where the incidence of malaria is relatively high.

Methods & Materials: The district administration has taken different innovative measures by organizing health – camps where the needed tests are conducted. Fever survey, awareness programmes at the village level, free transportation of the malaria positive persons for treatment, distribution of medicines freely, specialist doctors to treat the Patients and free supply of food to the patient’s families besides initiating preventive measures like spraying ACM 5%. (Irs spray), Supply of mosquito nets (LLIN) and mosquito coils. RTD KITS, Micro Slides, Microscope, JSB 1, JSB 2, Methy Alcohol, M1, M2, M3, M4 Records, Anti larvals, ACT KITS, Cholorquine - Primaquine.

Results: The study analyses the cases of malaria in the district between 2001-2015 (up to September), extending over a period of 15 years. There are ups and downs in the number of cases though the maximum is 4811 in 2010 and the minimum is 803 in 2003. More over, infected migrants to the district from the border states are high during November – February.

Conclusion: Community as a whole has been motivated to tackle the challenge of malaria. All the measures of the district authorities are depicted in this paper along with suggestions.
Genotypic, phenotypic and functional profiles of NDM harboring Carbapenem resistant Escherichia coli from India

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Background: The emergence of Carbapenem resistant E. coli has been established as a major public health threat and represents a new challenge in the treatment of infectious diseases. India has been implicated in the transmission of NDM1 E. coli across the continent. It is therefore pertinent to investigate the current prevalence and characterize Carbapenem resistant superbugs in India.

Methods & Materials: A total of ~510 isolates were screened for Carbapenemase production followed by PCR amplification and sequencing of NDM genes. Antibiotic sensitivity towards 20 different antibiotics was performed using disk diffusion technique. MIC was determined based on HI-comb MIC test. Conjugal transfer was performed either by broth mating or filter paper based method. Adhesion, invasion, biofilm formation and serum resistance assays were performed to assess in vitro virulence properties. Isolates were genotyped by ERIC-PCR fingerprinting.

Results: Thirty nine (8%) out of the 510 E. coli were found to be Carbapenem resistant, of these 94% of the strains were positive for NDM gene. They comprised mainly (75%) of NDM-1 allele while other NDM types like NDM-5, NDM-7 and NDM-4 were also detected in rest of the isolates at varied proportions. NDM positive E. coli demonstrated higher resistance rates to both β and non-β-lactam antibiotics in comparison to the non-ESBL producing E. coli. In vitro conjugation suggests NDM transmission via plasmids. Particularly, FIA and FIB types of plasmids were identified in NDM positive E. coli. The NDM strains did not exhibit close genetic relatedness nor did they possess any specific virulence profiles. However, virulence assays underlined moderate pathogenicity of these isolates that could enable them to cause infections and protect themselves of different environmental stresses and insults.

Conclusion: Our study demonstrated that NDM-1 was the most prevalent metallo-beta-lactamase among Carbapenem resistant E. coli isolates in India, and demonstrated that there are no endemic NDM E. coli clones currently represented in our collection. Nevertheless horizontal gene transfer (via plasmids) was identified to be the potential mechanism for the spread of NDM genes. Therefore, early detection and surveillance of NDM-1 producing E. coli is urgently needed to prevent epidemic spread.
Background: Bluetongue is a vector-borne disease of ruminants caused by bluetongue virus (BTV) belonging to Reoviridae. So far, 28 serotypes of BTV have been reported, and distribution of various serotypes is not uniform among different geographical areas. The virus is endemic in tropical areas where vector population is abundant and these areas act as source population for virus spread to the neighbouring temperate sink areas. Australasia is one of the stable source-sink episystems with circulation of different serotypes. During this study, genetic relations of the serotypes circulating in the Australasian region were analysed and the serotypes that are unique to this episystem and those which entered this episystem during recent past were identified.

Methods & Materials: Data about BTV serotypes circulating and genome sequence were collected from public databases. Phylogenetic analysis was done using MEGA and BEAST.

Results: Majority of the BTV serotypes circulating in Australasia are either unique (BTV-20, -21 and -23) to the region or distinct (BTV-1, -2, -3, -4, -9 and 16) from their counterparts in other source-sink episystems and belong to Eastern topotype. Apart from these, serotypes of western origin are also found to be circulating (BTV-2, -5, -7, -10, -12 and -24), and some (BTV-2 and -10) are indistinguishable from the live attenuated vaccines being used in Africa (BTV-2) and USA (BTV-10). However, these two serotypes are not reported outside India.

Conclusion: We could conclude that BTV-20, -21 and -23 are unique to Australasian region and eastern topotype viruses of serotypes BTV-1, -2, -3, -4, -9 and -16 have probably diverged from their western counterparts hundreds of years ago and evolved since then. Among the serotypes of western origin, BTV-5, 7, -12 and -24 seem to have entered this area in the recent past and got established in more than one country whereas vaccine strains of BTV-2 and -10 did not. Identification of circulating serotypes of BTV in this region is important for evaluating virus movement and for vaccine design.
Background: Indonesia reports the second highest dengue disease burden in the world. However these passive surveillance data are recognized to be incomplete, to vary widely within the country, and are likely significant underestimates. Age stratified seroprevalence data are relatively unbiased indicators of past exposure and allow understanding of transmission dynamics. These information are valuable for public health planning, control and prevention activities. A nationally representative population-based cross sectional dengue seroprevalence study was conducted in 1 to 18 years old urban Indonesian children.

Methods & Materials: Using a cluster design and a probability proportional to size sampling, 3210 children from 1 to 18 years old in 4 age groups (1–4; 5–9; 10–14 and 15–18 years old) were enrolled, following household visits, from 30 clusters distributed from west to east in urban areas of Indonesia. Serum samples were tested for anti-dengue IgG antibodies by indirect ELISA. Using linear and catalytic models the median age of seroconversion and the force of primary infection were estimated.

Results: Data from 3194 children (98.7%) were included in the analysis. Overall, the adjusted national seroprevalence was 69.4% [95%CI: 64.4-74.3] ranging from 33.8% [95%CI: 26.4-41.2] in the 1-4 year old, 65.4% [95%CI:69.1-71.7] in the 5-9 years old, 83.1% [95%CI: 77.1-89.0] in the 10-14 years old, and 89.0% [95%CI: 83.9-94.1] in the 15-18 years old. The median age of seroconversion was 4.8, and considering a constant force of infection we estimated 137 primary infections per 1000 children per year.

Conclusion: This is the first nationally representative dengue seroprevalence study conducted in Indonesia. Dengue seroprevalence is high in Indonesian children: over half of children have been infected by the age of 5. This level of transmission intensity should be associated with increased risk of secondary infection and thus clinically and more severe disease. These data are an additional indicator of national-level burden and will inform implementation of preventive measures, including vaccination.
Background: A 7.6 magnitude earthquake on April 25, 2015 in Nepal followed by a series of aftershocks claimed almost 9,000 lives and caused injuries among 23,000 people. Tribhuvan University Teaching Hospital (TUTH), a tertiary care center, was one of the major hospitals in Kathmandu providing care to the earthquake victims. This study was conducted to identify etiological agents of various infections among earthquake victims admitted to TUTH.

Methods & Materials: A total of 357 samples were received from earthquake victims in the Microbiology laboratory of TUTH from 25 April to 1 July, 2015. The samples included pus/swab (n=130), blood (n=81), urine (n=77), sputum (n=47) and body fluids (n=22). These samples were received from emergency department and inpatient wards including intensive care units. Standard methodology were followed to identify the microorganisms and susceptibilities were done using disk diffusion method.

Results: Microbial growth was seen in 67.7% of pus, 18.2% of body fluid and 13.6% of blood samples. Similarly, significant growth was found in 48.9% of sputum and 28.6% of urine samples. Mixed growth of microorganisms was seen in 20.8% of pus, of which combined growth of *Staphylococcus aureus* and *Escherichia coli* was most common (n=3). *Escherichia coli* was the most common isolate from pus (n=26), followed by *Staphylococcus aureus* (n=18) and *Acinetobacter calcoaceticus baumannii (Acb) complex* (n=17). *Escherichia coli* (n=11) was the predominant isolate from urine samples. In sputum samples, *Klebsiella pneumoniae* (n=7) was most common followed by *Escherichia coli* (n=4) and *Pseudomonas aeruginosa* (n=4).

In blood cultures, *Citrobacter freundii* (n=3) and *Burkholderia cepacia* complex (n=3) were the common isolates. *Escherichia coli*, *Klebsiella pneumoniae* and *Acb* complex were isolated from different body fluid samples. Out of total 140 gram-negative bacterial isolates, 56.4% (n=79) were carbapenem resistant. Similarly, methicillin resistance was seen in 23.8% (n=5) of total 21 *Staphylococcus aureus* isolates.

Conclusion: Based on the specimens received by Microbiology laboratory, pus followed by blood and urine were the most common samples received and wound related infections were the most common type of infection among the earthquake victims. A variety of bacteria was isolated from these samples including a significant number of carbapenem-resistant gram negative bacilli.
Assessment of fourteen days primaquine treatment efficacy in plasmodium vivax malaria at primary and tertiary care centers in Southwestern India  
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Background: Acquaintance is scanty on PQ efficacy and P. vivax relapse in Udupi district, Karnataka, India. We assessed the efficacy of 14 days PQ treatment for preventing recurrence of P. vivax infection.

Methods & Materials: Microscopy and PCR proven P. vivax infected adults (≥18 years) from one tertiary and five primary health centres, pre-enrolled in a chloroquine-primaquine (CQ-PQ) combined therapeutic trial, upon convalescence on 28th day were requested to participate in another 15 months long follow up study. Participants were treated previously with CQ 25 mg/kg body weight over 3 days and PQ 0.25 mg/kg body weight daily for 2 weeks upon confirmation of G6PD levels. A complete adherence for the prescribed CQ-PQ regimens was noted. A peripheral blood smear examination was performed with every participant within 1–2 months duration. A positive P. vivax case was considered as relapse/reinfection and retreated with CQ-PQ. Data were analysed by independent t-test or Mann Whitney U test, χ² test or Fisher’s exact test and Cox regression using SPSS v15.0, South Asia, Bangalore, India.

Results: Of total 323 participants in CQ-PQ therapeutic trial, 114 participated in 15 months long follow up study. Of 114 participants, 28 (24.56%) recurred subsequently, including two participants with two recurrences and one participant with three recurrences. One patient did present with P. falciparum malaria after 3 months. The median duration of first recurrence was 3.14 months (IQR, 2.23 – 6.03) which ranged from 1.22 to 15.07 months. There were no clinical dissimilarities (p>0.05) among recurrence and non-recurrence groups. Participants with past history of P. vivax malaria had significantly higher odds of recurrence [HR (95% CI): 2.62 (1.24-5.54), p = 0.012]. The severity of disease (11.40%, 13/114) was not associated (p=1.00) with recurrence. Of 28 recurrent cases, 3 (10.71%) had severe malaria initially, however, none developed severe malaria during recurrences.

Conclusion: Despite complete adherence to 14 days PQ regimen, P. vivax results in substantial recurrences in Udupi taluk. Further, molecular investigations are required to determine the true relapse/reinfection proportion and their determinants. Patients with past history of P. vivax malaria are at high risk of recurrences.
Ethiopian dracunculiasis eradication, the end game challenges, 2015
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Background: Since a global campaign to eradicate dracunculiasis (Guinea worm disease) was begun in 1986, the number of cases has decreased from 3.5 million to 15 in 2015. The revised 2009 target date for eradication has passed. The disease transmission is currently limited to only South Sudan, Mali, Chad, and Ethiopia. Ethiopia started the campaign in 1994 and has decreased cases from 1252 to 2 in 2015. We analyzed surveillance data to better understand epidemiology of the disease and determine what sustaining transmission in Ethiopia.

Methods & Materials: Descriptive analysis of Guinea worm (GW) surveillance data from health facilities to Ministry of Health. A GW patient is defined as an individual exhibiting a history of a skin lesion with emergence of worm. Case containment is defined as prevention of transmission from individual patient. Data from 1994-2015 were analyzed using Microsoft Excel.

Results: Of the 3536 cases (196 per year), 2.7% were imported from South Sudan. Equal number of males and females were affected in 1996 and 2002, when 60% of 225 patients in 1996 and 82% of 39 patients in 2002 were 15-45 years old. There is year-round transmission of GW with incidence peaked from April to July (when 2499 of 3536 cases were reported). The incidence rate declined from 2.34 /100,000 in 1994 to 0.01/100,000 in 2015. The trend of number of villages reporting cases paralleled that decline. In 2008, after 20 months of zero reports, 41 cases were rediscovered. In 2010, 2011, 2012, 2013, 2014 and 2015 the case containment rates were 90%, 100%, 50%, 57%, 67% and 100% respectively. Since 2013, Ethiopia has started to detect Dracunculus medinensis infection in animals (17 dogs and 3 baboons) in same villages where human cases have occurred.

Conclusion: Although the number of cases and transmission has decreased significantly, the struggle to eliminate the disease remains a challenge, possibly because of inadequate case containment or undetected cases. This unusual epidemiologic pattern of GW is unlike that seen previously in Ethiopia. Heighten surveillance to detect and contain all cases to stop transmission, and study to characterize the peculiar epidemiology of the GW.
Factors associated with Ano-genital warts occurrence among Human Immunodeficiency Virus (HIV) infected patients in Gauteng, South Africa
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Background: The study highlighted factors associated with the occurrence of ano-genital warts (AGWs) among HIV infected individuals in South Africa (SA). Human Papilloma Virus (HPV) infection occurs more frequently among HIV infected individuals because of their compromised immuno-status.

Methods & Materials: We conducted a secondary analysis of routine clinical data from Ward 21 clinic in Hillbrow, Johannesburg. Ward 21 is one of the largest non-hospital ART initiation sites in the world and sees over 4000 patients monthly. Participants were patients ≥16 years who attended the ART clinic between 2009 and 2011. Bivariate analysis was done using Chi-Squared test. Logistic regression was conducted to assess factors associated with AGWs. Analysis was stratified by gender since it showed to be an effect modifier and we assessed the following factors: sexually transmitted infections (STIs) (syphilis, herpes simplex virus type 2 and scabies), age, first CD4 , employment-status and ART-status.

Results: AGWS prevalence was 4% and 3% among females and males respectively. The adjusted-odds of having AGWs among females above 25 years but below 55 ranged from 1.6 -18.3. A CD4 count of < 200 cells/ml³ was associated with AGWs (OR: 1.32; 95% CI: 1.02 - 1.72) amongst females. Among males the adjusted-odds of having AGWs if a patient was not on ART was 1.53 ;( 95% CI: 1.01 – 2.31).

Conclusion: AGWs were prevalent among younger age-groups indicating young people especially females are still getting exposed to HIV and STIs in their teenage years. More need to be done to reduce STIs in order to fulfill the SA government’s strategic objective of reducing STIs.

Age was strongly associated with AGWs among females. Recurrent AGWs have been found to be associated with older age however we could not establish if these were recurrent. ART-status was strongly associated with AGWs among males. This confirms that though the role out of ART in SA has increased in coverage a meaningful impact on AGWs is yet to be seen.

Going forward, conducting primary research will allow for collection of information on other relevant covariates. It would also be ideal to use an STI clinic database.
A multicentric surveillance of invasive pneumococcal disease (IPD) in children under five years in India

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**Background:** Invasive infections caused due to *S. pneumoniae* continue to be a major cause of morbidity and mortality among children under 5 years of age in India. The aim of ASIP surveillance was to generate nationwide epidemiological data on IPD, serotype distribution and antibiotic resistance pattern among *S. pneumoniae* isolated from children less than 5 years of age.

**Methods & Materials:** The study included 18 hospitals/institutions, 52 sentinel doctors and 10 sentinel microbiology laboratories from different territories in India with central reference laboratory located at CMC, Vellore. Children aged between 2-60 months suspected of IPD were recruited both prospectively and retrospectively and their sterile body fluids were investigated for the presence of *S.pneumoniae*. At the central reference laboratory, the submitted isolates were reconfirmed and serotyped using Quellung. The antimicrobial susceptibility testing and determination of MIC by E-test was performed as per established protocols.

**Results:** Of 361 patients identified both prospectively and through lab surveillance 132 (58%) presented with pneumonia, 78 (35%) meningitis, and 16(7%) had other clinical syndromes. Mortality was 3% overall with 8.0% among IPD cases. Although, 56 unique serotypes were found overall, 72% of all IPD were caused by 10 serotypes. Serotype 14(14.4%), 1(13.57%), 5(10.25%), 19F (9.1%), 6B (6.37%) and 19A (4.9%) were the most common with top 4 STs accounting for 47% of both pneumonia and meningitis cases. Penicillin and cefotaxime non-susceptibility was 5.3% and 1.0%. Non-susceptibility to cotrimoxazole and erythromycin resistance was highest with 35.4% and 60.5%. Overall, PCV coverage rate was high for PCV13 (74%, 95%CI (69-78%)).

**Conclusion:** ASIP study has provided surveillance data on IPD covering major geographical regions in India. Currently available and licensed vaccines would provide good protection coverage in Indian children against IPD.
An analytical study of behavioral risks and illness among camel keeper and non-camel keeper at zoo parks in Thailand 2014
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**Background:** Since 2012, a novel corona virus, Outbreak of Middle East Respiratory Syndrome (MERS) has been emerged in many parts of the world. Surveys have shown the evidences of neutralizing antibodies in samples from camel in Arabian Peninsula coincided with at the same areas where most of human cases were reported. Camel raising was found in some areas of Thailand, especially in zoological parks. The objective of study was to determine behavioral risks and illness among zookeepers.

**Methods & Materials:** A cross sectional study was conducted in seven zoological parks in 2014. Zoo Keepers were divided into two groups; a group of camel keepers and non-camel keepers. Face to face interview was conducted to obtain information regarding history of animal exposure, use of personal protective equipment (PPE) and history of illness. Univariate analysis and multiple logistic regressions were performed to describe the relationship of illness and behavioral risks for zookeepers.

**Results:** From 50 study populations in this study, 59.50% of zoo-keepers reported regular wearing boots at work, but wearing mask, apron and gloves were low. Four camel keepers and one non-camel keeper reported illness during the study period and sought treatment at hospitals. One of the camel keepers, who also taking care of alpaca, was admitted to the hospital with diagnosis of influenza. Epidemiological evidences suggested that he was likely exposed to infection from his daughter, who had influenza like illness a week before his illness. There was no statistically significant difference of illness between camel keeper and non-camel keeper, and there was no statistically significant difference of illness and behavioral risk factors.

**Conclusion:** Surveillance in a group of close contact with animal should be developed. Effectiveness of enforcement and measure about the use of PPE and training course to provide perceptions of health threats and benefits gained by wearing PPE should be implemented at the zoo parks. With these measures, they can reduce risk of emerging infectious diseases among people having close contact with animals.
Outbreak of suspected cholera associated with unprotected well water, Biral B village, Gulbarga district, Karnataka, 2015
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Background: Most cholera outbreaks are due to faecal contaminated drinking water. On 25 April 2015, Gulbarga district of Karnataka reported 82 diarrhoea cases in Biral B village. We conducted an investigation to identify risk factors for illness and to provide recommendations to control the outbreak and prevent future illness.

Methods & Materials: We defined a suspect case of cholera as ≥ 3 loose stools in 24 hours in a resident of Biral B village between 19 April and 7 May, 2015. We identified cases by active surveillance through a house to house survey. We conducted a retrospective cohort study in every third household. We interviewed 565 persons in 177 households to assess illness status, socio-demographic characteristics, and potential risk factors including water sources and water treatment. We calculated relative risk (RR) and 95% confidence interval (CI). We collected five stools samples for testing of *Vibrio cholerae* at district referral laboratory. We assessed water sources, water distribution and tested all sources for faecal coliforms.

Results: We identified 169 cases among 2495 villagers (attack rate = 7%). Three fourths (126) were hospitalized with no deaths. Illness onset dates ranged from 22 April to 7 May 2015. The median age was 25 years (range 1-85yrs) with the highest attack rate of 14% (33/235) among 26 to 35 years group and the lowest attack rate of 1.4% (8/561) among the age group 46 to 55 years. The attack rate was 16% (14/86) among persons using water from an unprotected, hand-dug well A for drinking or cooking (RR: 2.2, 95% CI: 1.2-3.8) compared to 10% (15/149) for a second unprotected hand-dug well B (RR: 0.8, 95% CI: 0.5-1.5) and 7% (28/380) for any of six tube-wells (RR: 0.6, 95% CI: 0.4-1.0). One of five stools samples was positive for *Vibrio cholerae* El Tor Ogawa by culture. Water samples from wells A and B, the six tube wells, overhead tank, and household taps had faecal coliforms and deemed not potable.

Conclusion: This suspected cholera outbreak was from using non-potable water particularly from one unprotected well A for drinking or cooking. We recommend chlorination, protection, and regular testing of water sources, particularly well A.
Hepatitis E outbreak among factory workers due to contaminated factory water, Mandya District, Karnataka, India, 2015

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Background: Hepatitis E virus (HEV) is the leading cause of acute viral hepatitis with over 2 billion cases per year. In India, HEV accounts for 60% of acute viral hepatitis cases. On 10 July 2015, Mandya district in Karnataka, India reported 50 cases of jaundice from factory A. We investigated to describe the outbreak, identify risk factors and propose recommendations.

Methods & Materials: We defined a case as acute onset of jaundice (yellow discoloration of eyes/dark coloured urine) in factory A worker between April and July 2015. We did a retrospective cohort study between 15th and 30th July. We interviewed workers about socio-demographic characteristics, food exposures and water sources. We calculated relative risk (RR) and 95% confidence interval (CI). We collected 19 serum samples for IgM testing by ELISA for Hepatitis A, Hepatitis E and Leptospirosis. We assessed water sources and collected water samples from canteen, overhead tank and river for testing faecal coliforms.

Results: We identified 132 cases between 25 May and 28 July 2015. There were no deaths, but most (73%) cases were hospitalised. Attack rate was 14% (132/950) and almost equal between genders (females 14% [114/812] and males (13% [18/138]). Median age was 29 years. Drinking water from factory tap (RR=1.7, 95% CI=1.2-2.5), eating lunch daily from factory canteen (RR= 2.1, 95% CI=1.4-3.1), or eating at least once from factory canteen in past three months (RR=2.4, 95%CI=1.7-3.3) were associated with illness. Using public water for drinking or cooking at home (RR=1.1, 95% CI=0.8-1.4) was not significant. Washing hands with soap after defecation was protective (RR=0.6, 95%CI: 0.4-0.9). Eighteen serum were positive for HEV. River water was filtered in sand filter and stored in overhead tank, but was never chlorinated. Factory tap water was used for cooking in canteen. Water samples from all three sites were positive for faecal coliforms.

Conclusion: This HEV outbreak in factory A in Mandya district was probably due to drinking water from factory and eating from factory canteen. We recommend providing safe water for drinking and cooking by changing sand in filters, chlorination and periodic testing of water. We recommend promoting hand washing practices.
Describing the interactive model design of avian influenza: Animal infection and human infection

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Background: Avian influenza is caused by H5N1 virus and commonly human are not infected because of the effective culminating procedure. Still the risk of infection to human is elevated due to increased contact with poultry and migratory birds. Case fatality rate of the infection increases the need to understand the dynamics of transmission of H5N1 from migratory birds to human through domestic poultry; study aims to illustrate the interactive model design of avian influenza and describing the points of interactions.

Methods & Materials: We considered the avian influenza migratory bird hypothesis as the cause of introduction of H5N1 virus in a susceptible population of poultry and human. In this study, we assumed SIR model for migratory/wild birds, SIC model for poultry and SIR model for human infection. For this study considered the recent outbreak of H5N1 in Kerala as a methodological sample and demographical, epidemiological factors from Kerala has been used for modeling purpose.

Results: Study results indicate the certainty of the migratory bird hypothesis for introduction of H5N1 in poultry. We also described the points of interaction between these three models. Mechanism of operation of the H5N1 interactive model design has been demonstrated with the projected population parameters.

Conclusion: This interactive model of avian influenza infection describes the risk of infection to human population and its transmission in a vulnerable human population. More epidemiological investigation is needed to explore the modeling pathways of the avian influenza in two species. Model design should be done with real ground level data from the veterinary department and health department so that the real crossovers can be identified and effective preventive measures could be implemented.
The impact of a SMS-based disease outbreak alert system (mSOS) in Kenya
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\textbf{Background:} Epidemic-prone diseases pose serious public health risks to African countries and populations around the world. In Kenya, similar to other African countries, compromised health systems hinder the compliance to Integrated Disease Surveillance and Response (IDSR) and International Health Regulation (IHR) policies, resulting in incomplete, delayed or poor quality paper based reports from the peripheral health facilities. Widespread expansion of mobile phone penetration and network coverage in Africa offers an opportunity to overcome communication and human resource challenges and potentially improve public health practice through mHealth. Small-scale projects are rarely rigorously evaluated which limits evidence-based policy adoptions and scale-up. This study addresses the scarcity of data from a randomized controlled trial in Kenya testing the effects of a real-time reporting of immediately notifiable diseases.

\textbf{Methods & Materials:} A cluster randomized controlled trial was undertaken between November 2013 and April 2014 in Busia and Kajiado counties in Kenya. A total of 135 health facilities were randomized into either the intervention group where health workers received training on mSOS and routine surveillance, or a control group that received only routine surveillance training. The mHealth intervention, mSOS (mobile SMS based disease outbreak alert system) is composed of formatted SMS communication between health workers and health managers (disease surveillance coordinators at the sub-county, county and national levels), and mSOS web based portal used for monitoring notifications. Health workers used mSOS text messaging system for 6 months to send patient-level information on suspected cases that require immediate notification. Messages were sent to a toll-free number set with a telecommunication provider in Kenya. Health managers received text message real-time on their mobile phones.

\textbf{Results:} The results showed that timely notifications were significantly higher in mSOS intervention group (+16.6\%, 95\% CI=2.71-25.07), which despite large improvements remained suboptimal with only one-fifth of detected cases notified.

\textbf{Conclusion:} This study showed promising potential of how innovative technology could help in increasing the notification rates of suspected priority diseases and enhancing International Health Regulations compliances in resource-limited settings.
Detection of mycobacteria in raw milk and assessment of risk factors among fulani herdsmen in Bwari Area Council, Abuja, Nigeria

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Background: Cross sectional study was carried out to detect mycobacteria in raw milk samples of from Fulani herds in Bwari Area council, Nigeria. ZN-stain technique, PCR and culture on LJ slants. Herd prevalence, knowledge attitude with associated risk factors for transmission of zoonotic TB were evaluated using structured questionnaires.

Methods & Materials: Study Area
Bwari area council (BAC) is one of the six area councils of the Federal capital territory. Located along coordinates 7° 8' E and 9° 24' N. Study Population: The pastoral Fulanis of Abuja are transhumant. They rear cattle, sheep, and goats. Sampling: Milk samples were collected by cattle owners into 50ml sterile falcon tubes as part of their routine milk collection, labeled and transported to the laboratory in cold chain. Samples were tested for M.bovis by PCR, ZN stain and cultured on LJ slants (figure 2).

Results: One hundred and forty five samples were analyzed. Positive detection rates by ZN-staining and culture were 6.89 % and 1.3 % by PCR respectively. Herd prevalence per satellite town by ZN-stain technique were 8.89 %, 10.00 %, 3.33 % and 5.00 % for Bwari, Dei-Dei, Kubuwa, and Ushafa respectively, while by culture they were 2.22 %, 10.00 %, 13.33 % and 5.00 % for Bwari, Dei-Dei, Kubuwa, and Ushafa respectively. Herd prevalence by PCR for Bwari was 2.22 % while that of Dei-Dei was 3.33 %. Kubuwa and Ushafa had 0 % prevalence each by PCR. Awareness level on cause and transmission mode of M.bovis from cattle to human through questionnaire revealed 64 % (11/17) and 82 % (14/17) were neither aware of the causative agent nor its zoonotic significance respectively. There was high risk of M.bovis transmission due to frequent raw milk consumption without boiling (OR = 5.76; CI = 1.32 – 25.18 and OR = 0.0947; CI = 0.0194 - 0.4619 ) with P < 0.05 respectively in the studied herds.

Conclusion: Mycobacterium bovis was detected in cow’s milk from Fulani herds of Bwari Area council, calls for implementation of public health control measures such as improved hygiene and milk pasteurization.
Impact of outpatient neuraminidase inhibitor treatment on hospitalisation in patients infected with influenza A (H1N1)pdm09: An IPD analysis

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Background: Neuraminidase inhibitors (NAIs) were widely deployed during the 2009 A(H1N1) pandemic. While evidence exists to support their effectiveness in reducing mortality in hospitalised patients, the impact of outpatient or community-based treatment on hospital admission has not been established. Our aim was to investigate the association between outpatient or community-based NAI treatment and admission to hospital in patients with A(H1N1)pdm09 virus infection.

Methods & Materials: We performed an Individual Participant Data (IPD) meta-analysis on a pooled dataset of 6,030 patients from nine different countries. ‘Admission to hospital’ was the main outcome and ‘NAI treatment initiated in the community or as an outpatient’ was the main exposure variable. We estimated Odds Ratios (OR) and 95% Confidence Intervals (95% CI) using generalised linear mixed modelling to account for clustering within each contributing study centre; and we adjusted for pre-admission antibiotics and NAI treatment propensity in the final model. To account for relatively frequent exposure to NAI treatment and an overall high likelihood of hospitalisation, we also estimated Risk Ratios (RR) using a random effects probit model.

Results: Of the 6,030 patients included in the pooled analysis, 5,738 (95.16%) had laboratory confirmed influenza A(H1N1)pdm09, and the remainder were clinically diagnosed with ‘pandemic influenza’. A total of 1,541 patients (25.56%) received outpatient or community-based NAI treatment and 4,250 (70.48%) hospitalisations occurred, indicating a population at overall high-risk of influenza-related hospitalisation. After adjustment for pre-admission antibiotics and NAI treatment propensity, outpatient or community-based NAI treatment was associated with a decreased odds of hospital admission (OR: 0.33, 95% CI: 0.28 to 0.39) compared to no NAI treatment. From our random effects probit model, we obtained a corresponding adjusted RR of 0.52 (0.48 to 0.57).

Conclusion: In a population with confirmed or suspected A(H1N1)pdm09, and at high risk of hospital admission, outpatient or community-based NAI treatment substantially reduced the likelihood of requiring hospital admission.
Spatial and temporal dynamics of the cases of tuberculosis in the zone of farming health of Pendjwa, Province of Bandundu/RDC, 2009-2013

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Background: Enclosed to the north extreme of the Province of Bandundu, the zone of farming health of Pendjwa (ZSR) is one most affected by the Tuberculosis (TBC) mainly at the Pygmy populations. This survey aims to identify the areas of health (AH) of persistence of the vestigial cases of TBC in order to propose adjustments adapted to struggle against the tuberculosis in this ZSR.

Methods & Materials: The cases of TBC returned between 2009 and 2013 to the scale of the AH as well as the individual cases returned in the structures of hold in charge have been analyzed.

Results: Three AH out of sixteen (Pendjwa, Nzale, Monio) have been identified like hot spotlight of the persistence of the cases of TBC. These three AH are populated from 55 to 60% of Pygmies. A total of 470 cases out of 535 had a notion of tubercular numbering in a brought closer environment.

Conclusion: The fine analysis of the persistence factors in these three AH would permit to bring the ZSR of Pendjwa to reduce the impact of the TBC to 83 for 100 000 habitants (half of the national yearly impact in 1990).
Prevalence and re-infection of Schistosoma mansoni among school children in Mekele town, North Ethiopia  
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**Background:** Although regular treatment of high risk groups is one of the control strategies of schistosomiasis, drug-based control programs are hampered by the continued susceptibility of treated individuals to re-infection. The aim of this study was to assess the prevalence and intensity of *Schistosoma mansoni* infection and re-infection among school children in Mekele Town of Tigray regional State, Ethiopia.

**Methods & Materials:** A school based longitudinal study was conducted from March to June 2011 in Mekele Town of Tigray regional State, Ethiopia. Fresh stool samples were collected from the study participating students and processed by Kato thick smear technique with three slides being prepared from each stool sample. *S. mansoni* positive individuals were treated with a single oral dose of 40 mg/kg of Praziquantel and those infected with other intestinal parasites were treated with Mebendazole for *Ascaris lumbricoides* and *Enterobius vermicularis* and Praziquantel for tanea species and *Hymenolepis nana* infections. Clearance of *Schistosoma mansoni* infection was confirmed four weeks after treatment of the positive individuals using Kato thick smear technique. Re-infection of *Schistosoma mansoni* was estimated after examining stool sample collected three months after the first Praziquantel treatment from those reported as being cured by the treatment based on the result of efficacy assessment.

**Results:** At baseline, overall prevalence of intestinal parasite was 30.6% and re-infection after three months post treatment was 28.8%. The prevalence of *S. mansoni* was 26.12% during the baseline survey (intensity of infection was 49.6 EPG) and 23.8% of those reported as being cured were re-infected (intensity of infection was 34.3 EPG). The efficacy of a single oral dose of 40 mg/kg PZQ four weeks after treatment was 82.4%.

**Conclusion:** The high level of *S. mansoni* re-infection as measured after three months of treatment implies that treatment of only positive cases may not have significant impact in the control of schistosomiasis in this endemic area.
Epidemiology of needle stick-sharp injuries (NSSIs) and potential high risk exposures among health professionals in Ethiopia: Neglected public health concern

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Background: Health professionals are exposed to a wide range of hazards in the workplace. Needle stick injuries have been recognized as one of the occupational hazards. Healthcare worker handling sharp devices or equipment is at risk of occupational exposure to blood borne pathogens. Despite the burden of potential exposures, in Ethiopia, there are only few researches that have been conducted; as a result there is clearly paucity of information on this regard. The aim of the research conducted was to determine the epidemiology of needle stick-sharp injuries and high risk exposures among health professionals in public hospitals, Addis Ababa, Ethiopia.

Methods & Materials: Hospital based cross sectional survey conducted among health professionals at public hospitals, Addis Ababa, Ethiopia. A pretested and structured questionnaire was utilized to collect data on socio-demographic, needle stick injury and other high risk exposures. Data was analyzed using SPSS version 16. Statistical significance was declared at P-value <=0.05.

Results: Of the total study participants, prevalence of sustained needle stick injuries (NSIs) and sharp injury was found 155 (61.2%) and 127 (50%), respectively. Majority of the study subjects, which account 184 (72.4%) and 153 (60.2%) of them were exposed for blood while ungloved and body fluid, respectively. Consistent use of gloves was reported by 52.4% of respondents. Of the total study participants, 9 (3.5%) of respondents were vaccinated against hepatitis B virus infection.

Conclusion: The study declared that exposure for potentially infectious body fluids including blood, needle stick injuries, sharp injury and other risk factors was high. But, the study indicated only very small percentages of health professionals were partially vaccinated for HBV. Taking into account the chance of potential exposure, there is a need to focus efforts on mitigating blood borne pathogen transmission through making the work place environment safe and making use of the available vaccine by vaccinating all health care workers at the start of their career.
Social, economic, and immunological impacts of TB treatment in Eastern rural China

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Background: Drug resistant TB in China has been rapidly growing and becoming a cause for critical public health concern. It was reported that the acquired drug-resistant TB mainly results from incompliance to treatment, uncompleted treatment, irregular treatment and unqualified health services.

Methods & Materials: Case-cohort was established in four counties in eastern rural China. All registered active TB patients from April 2013 to March 2014 were investigated using structure questionnaire to collect demographic, geographic, socioeconomic information and disease profile. Enzyme-linked immunosorbent assay (ELISA) was used to assess the level of serum cytokines (IFN-γ, IL-4) before anti-TB treatment. All the participants were followed up 6 or 8 months until treatment was completed.

Results: In total, 1404 patients were recruited, in which 1270 (94.5%) were newly diagnosed TB patients, 447 (33.8%) were smear-positive (SS+). The average age was 49.5±18.8, and 76.2% was male. The serum IFN-γ and IL-4 before anti-TB treatment was 7.936 and 12.292 pg/ml respectively. 80.9% of newly diagnosed TB patients received standard anti-TB treatment, while 87.7% retreatment TB patients received standard anti-TB treatment. The successfully treated rate was 92.5% and 78.6% of new TB patients and retreatment TB patients respectively. The sputum conversion rate of SS+ TB patients after 2 month anti-TB treatment was 96.5%, while the cure rate and successful rate was 80.5% and 90.0% respectively. 92.6% smear-negative patients completed anti-TB treatment. The successful rate in Jiangsu (97.3%), the more developed area, was higher than in Jiangxi (87.6%) (OR = 6.276, 95% CI: 2.528 15.579). Patients with BMI<18.5 may be more risk to fail the treatment compared with the health BMI (18.5-23.9) patients (OR = 3.196, 95% CI: 1.592 6.418). There was no relationship between serum cytokines level and treatment outcomes. The cure rate of TB comorbidity with diabetes was same as the one without diabetes.

Conclusion: The sputum conversion rate, cure rate and successful rate of TB patients were high in eastern rural China. Improving patients' nutrition, keeping a healthy BMI could be the key point of increasing successfully treated rate of tuberculosis. More research should be done in future to evaluate the impact of the serum cytokines during anti-TB treatment.
Incidence and risk factors for Tenofovir induced nephrotoxicity among patients with HIV on stable combination antiretroviral therapy (c ART) in South India

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**Background:** Tenofovir disoproxil fumarate (TDF) is bioavailable prodrug of Tenofovir, a potent nucleotide reverse transcriptase inhibitor. TDF causes Proximal tubular dysfunction and Fanconi's Syndrome. Number of patients on TDF in India is rising due to decrease in cost and availability of TDF in both first and second line antiretroviral therapy (ART) regimen through National ART programme. TDF related renal toxicity in our population has not been studied systematically. Hence this study was undertaken.

**Methods & Materials:** This descriptive study was done on HIV positive patients on stable cART containing TDF with normal Renal function at baseline, attending ART centres under KMC Hospital, Mangalore from September 2012 to Dec 2013. Data was collected by semi structured proforma which included sociodemographic profile, duration of HIV and cART, CD4 count, body weight, BMI, creatinine, GFR using Cockcroft-Gault equation. Using RIFLE Criteria (Risk, Injury, Failure, Loss, End stage) by Acute Dialysis Quality Initiative Group, patients were divided into different stages. Data was analysed using SPSS Version 11.5.

**Results:** 63 patients who met inclusion criteria participated in this study. 35 were male and 28 female. Median duration on TDF was 10 months (7-14 months). 25 (39.68%) developed renal failure according to RIFLE Criteria. 21 (84%) out of these had Risk, 2 had Injury, 1 Failure and 1 Loss. 22 patients out of 56 (36%) on NNRTI based regimen and 3 out of 7 (43%) patients on Protease Inhibitor based regimen had Renal Toxicity. 15 (60%) of patients with renal toxicity had weight less than 53 kg (p=0.001). 16 (64%) had CD4 Count less than 275 cells/ml (p=0.02). 12 out of 28 females (42%) and 13 out of 35 males (37%) (p=0.01) had renal toxicity.

**Conclusion:** TDF induced renal toxicity is high, though majority of patients had mild toxicity. Low body weight, female sex, Low CD4 Counts and Protease Inhibitor based cART are risk factors for renal toxicity. Frequent monitoring of renal functions is advocated among patients with these risk factors who are started on TDF.
Variations in the elimination of new HIV infections among children in Africa

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**Background:** Sub-Saharan Africa is responsible for the majority of new HIV infections among children. An initiative was launched in 2011 to rapidly eliminate new HIV infections among high burden countries. This research evaluated the progress and challenges of two indicators of Global Plan among 21 sub-Saharan priority countries.

**Methods & Materials:** Data from Joint United Nations Programme on HIV/AIDS 2014 Progress Report on Global Plan was used to determine the differences among the Eastern/Southern African (ESA) and Central/Western African (CWA) countries in terms of the number of new paediatric infections and mother to child transmission (MTCT) rate. Published literatures were systematically reviewed from January 2011 to September 2015 using 3 databases; Scopus, Pubmed and ISI Web of Science with respect to the challenges faced by these countries in eliminating new HIV infections among children.

**Results:** A total of 214,700 infants and children were newly diagnosed with HIV infections in 2013, 135,800 (63%) children were from ESA while CWA had 78,900 (37%). In 2013, the MTCT rate among the ESA and CWA countries were 15% and 26% respectively; p= 0.0051. The MTCT rate decreased from 11% in 2009 to 6% in 2013; p= 0.0434. A total of 16 peer reviewed articles were included in this review after a comprehensive search of databases. Challenges being encountered by these 21 countries included low HIV testing rates during pregnancy, poor adherence to antiretroviral therapy, poor linkage between mother—child pairs and post-natal healthcare services, delayed and low early infant diagnosis coverage, low paediatric ART uptake, and high unmet needs for family planning service among the women living with HIV.

**Conclusion:** Both the final MTCT rate and the number of new infections reduced progressively between 2009 and 2013 but the performance are still below the expected milestone. The performance of ESA countries was better when compared with the CWA countries. There is need to put more effort in the bid to eliminate new infections among the countries and the two regions to must collaborate and share experiences.
Changing the content of Cl, Ca, K, Na in the hair of HIV-infected patients depending on the concentration of CD4 lymphocytes

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**Background:** A study of the imbalance of macro elemental composition of the HIV infected body is necessary for the understanding of the essence of pathochemical function of the immune system. The purpose of the study is to perform the assessment of certain macro elements in the hair of HIV-infected patients.

**Methods & Materials:** The research examined the macro elemental composition of the hair in 46 HIV-infected patients, 20 healthy individuals, aged 21-54 years, who were at different stages of the infection. All studied patients with HIV infection were registered at the Republican Centre for AIDS Control and Prevention with a confirmed diagnosis. Hair neutron activation method was used to study macro elements in all patients. Patients with HIV infection were divided into 3 groups depending on the concentration of CD4 lymphocytes: group 1 patients had CD4 lymphocytes concentration >500 cells/μL; group 2 - concentration of CD4 lymphocytes between 200-499 k/μL; and group 3 - concentration of CD4 lymphocytes <200 cell/μL.

**Results:** The concentrations of Cl, Ca, K were lower in all three groups regardless of the concentration of CD4 lymphocytes. Cl concentration was decreased in all three groups, but the greatest reduction of a factor of two was observed in patients with AIDS. Concentration of Ca has been reduced by half. Na was reduced in 76% of HIV-infected patients, with the largest decline noted in the third group (260 ± 80) (P <0.01). An average decrease in K (140 ± 15 μg/g) by 2.7 times was observed in all three groups of HIV-infected patients; meanwhile, just in the group 1, the potassium concentration decreased by a factor of six.

**Conclusion:** The studies have shown changes in macro elemental composition in the hair of HIV-infected patients. Macro elemental imbalance, which was found in HIV-infected patients in this study, affects the function of the immune system as well as other body systems.
Private public partnership for stigma free HIV service delivery in APAIDSCON network in India

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**Background:** Andhra Pradesh state has the highest HIV burden in India. Andhra Pradesh AIDS Consortium (APAIDSCON) the largest public health partnership a network of 20 medical colleges established to address HIV AIDS in India was spearheaded by SHARE India and funded in part by United States Centers for Disease Control & Prevention Cooperative agreement # U62/CCU025160-02. 2005 –2010

**Methods & Materials:** Results of Interventions to reduce stigma and discrimination were analyzed. Both quantitative and qualitative methods were used. Adapted questionnaire on stigma and discrimination was used. Methods included In Depth Interviews with key stakeholders and patients to understand reporting of Stigma & Discrimination. Focus Group Discussions were used. Ethics committee approval was obtained.

**Results:** The qualitative data indicated that stigma and discrimination present in the consortium among Health Care Providers due to the “fear element” was substantially decreased due to measures taken by the project like training and sensitization programs for HCPs, guidelines and protocol implementation to reduce stigma and discrimination, prevention of segregation i.e. separate bed or special identification marks during admission, staff compensation and facilitation of universal precautions. The qualitative data demonstrated that management of the sampled private hospitals never placed hurdles to admitting HIV positive cases. This created a conducive environment against stigma and discrimination. Instructions were given not to mention HIV positive status in patient's case record. Segregation of HIV positive patients for routine care was eliminated.

After conclusion of the project follow up interviews of 117 in-patients from four of the sampled institutes demonstrated persistence of best practices with more than 96% of the PLHIV responding that there is no differential treatment or denial of treatment in these sites.

**Conclusion:** Across the APAIDSCON consortium persistent efforts to remove the knowledge barriers had a salutary effect; HIV services could be provided without stigma and discrimination in the private sector. Grounded efforts with the managements both in private and public sector yield sustained results long after the intervention ended.
Insilico analysis of micro RNA based target interaction associated with pharmacogenomics of acquired immune deficiency syndrome
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Background: Analysis of micro RNA(miRNAs) based regulatory networks in infectious diseases is a challenging task in the era of post genomics and prediction of the miRNAs associated with pharmacogenomics of acquired Immune Deficiency Syndrome (AIDS) is an initiation towards the miRNA based therapy for AIDS.

Methods & Materials: Initially we have taken the list of human genes associated with AIDS from PharmGKB and further we have analyzed the network of human miRNAs which are associated with AIDS related genes from EnrichR. Finally we performed statistical analysis like P value, Z test and combined score of Students T test and ANNOVA to analyze the significance of those predicted miRNAs towards the Human Immune Virus (HIV) related genes.

Results: PharmGKB contains 6 genes (ABCB1, CYCSP5, G6PD, GSTM1, NAT2 and NR1I2) which are associated with the Pharmacogenomics of AIDS. EnrichR has predicted 6 miRNAs (hsa-miR-18a, hsa-miR-1, hsa-miR-206, hsa-miR-24, hsa-miR-125a and hsa-miR-125b) which are related to AIDS related genes. Based on the statistical analysis, it has been found that hsa-miR-18a has a minimum P value and Z score of 0.0370 & - 1.82 respectively. Association of hsa-miR-18a with HIV related genes shows a 4 fold increase when compared with hsa-miR-1 and hsa-miR-206. Similarly the association of hsa-miR-24 shows a 3 fold decrease when compared with hsa-miR-18a and finally, hsa-miR-125a and hsa-miR-125b has a 5 fold decrease when compared with hsa-miR-18a.

Conclusion: Based on the statistical analysis of gene and miRNA regulated networks, it was found that hsa-miR-18a show a good statistical significance towards genes which are related to AIDS and in future, hsa-miR-18a may turn out to be a potential target for the miRNA based therapeutics of AIDS. At present, we have applied the above mentioned methodology for AIDS and in future, this methodology will also be applied to other infectious diseases.
The elements of paediatric HIV status disclosure: A qualitative study from Karnataka, India
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**Background:** With improved access to combination anti-retroviral therapy (cART), children with HIV are growing into adolescence and adulthood. If the children are deprived of the knowledge regarding their disease, there would be significant challenges in coping with the disease. Hence this study was conducted to assess the various elements of the disclosure of paediatric HIV status.

**Methods & Materials:** Focus group discussions were conducted in June 2015 among the caregivers of HIV positive children visiting an Anti-Retroviral Therapy (ART) centre in a Southern coastal city of Karnataka, India. Snowballing method was adopted for recruitment of the caregivers. The groups consisted of 8 members. Anonymity was maintained by giving codes for the participants. The discussions were conducted in Kannada, the official language of Karnataka. The discussions were voice recorded, transcripts prepared in English and analysed. Written informed consent was taken from each participant and Ethical committee clearance was obtained for the study.

**Results:** All the caregivers opined that the children have to be informed about their HIV status mainly for their knowledge and better adherence to self-care and medications. The caregivers felt that they themselves have to tell the children at the comforts of their homes, when the child was around 7-10 years of age. A majority of the participants felt that the children need to be told as a process over time and not as a discrete one-time event. They would also appreciate the help of the counsellors, especially in answering the children’s questions. Although improved adherence was seen in the older children, there were no benefits among the younger children. The negative aspects of disclosure commonly seen were negative emotional reactions like sadness, worry or anger which subsided over time. The children did not face any stigma or discrimination following disclosure.

**Conclusion:** As there are more benefits than harm from disclosing the HIV status to the child, disclosure should occur as a slow evolving process over time starting around 7-9 years and done preferably by the caregivers, assisted by the counsellors. Development of culturally appropriate interventions to facilitate disclosure of HIV status to infected adolescents is key to improve retention, adherence and other outcomes.
Development of engineered nanocarrier for controlled delivery of a protease inhibitor
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Background: AIDS is a chronic, progressive syndrome, characterized by intense viral replication and profound immunosuppression, resulting in the development of life threatening opportunistic infections. HIV infection leads to deterioration of immune functions.

The objective of the present study was to develop, optimize and characterize engineered nanocarriers for controlled delivery of a protease inhibitor. Lopinavir was the drug of choice as it is an effective antiretroviral drug having specific and prominent anti-HIV action. In the present study, it is envisaged to develop and characterize a controlled delivery system wherein the drug lopinavir (LPV) will be entrapped in engineered nanocarrier. Engineered nanocarriers targeted towards the prespecified target tissues by coupling with mannose delivers the drug in a controlled manner to the site of action. Thus it results in increased bioavailability and avoids the adverse effects associated with the drug. Overall the approach leads to a safe, economical and effective Anti-HIV formulation.

Methods & Materials: The uncoupled Solid Lipid Nanoparticles (SLN) were prepared by Solvent diffusion method and then coupled with mannose. Characterization studies were done by Scanning & Transmission Electron Microscopy (SEM & TEM). X-ray diffraction (XRD) and Differential scanning calorimetry (DSC) studies were performed along with the in-vitro studies followed by in-vivo studies on albino rats.

Results: In-vitro & in-vivo studies results shows Mannose coated SLNs (MSLN) deliver their contents to macrophage rich organs and tissues, which are the reservoir of HIV. Low elimination and better distribution profile can be achieved by MSLNs. The dose of the antiviral agent can be reduced due to the site-specific delivery from this carrier.

Conclusion: Conclusively, ligand-mediated bio-disposition and cellular interaction of MSLNs, especially at the target sites, would be a focal paradigm for upcoming research in the field of anti-HIV drug delivery. MSLNs have paved the way for the bio-stable, site-specific and ligand-mediated delivery systems with desired therapeutics.
Prevalence and risk factors associated with immunological non-response in HIV-1 infected patients treated with NNRTI based first line drugs in South India

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Background: The absence of a recovery in the CD4+ T cell count during long-term, virologically suppressive HAART is an unquestionable source of anxiety to HIV-infected patients and their treating clinicians, given the long-term risks of disease progression and death, underscoring the need for means of identifying early predictive factors and treatment options.

Methods & Materials: To study the prevalence, risk factors associated to immunological non-responders (INR), and T-cell recovery pattern, we cross-sectionally analyzed 522 HIV patients on NNRTI based first line HAART at 6th month and 12th month during therapy.

Results: Among 522 HIV patients, we found 56 (10.72%) each failed to achieve at least 50 cells after 6 months and 100 cells after 12 months of HAART. Lower baseline viral load (median 4.4 log 10 copies, IQR 3.80 – 4.88, p <0.0001), higher baseline CD4 count (median 309.5 cells/µL, IQR 229.2 - 386.7, p <0.05), high nadir CD4 count (median 260 cells/µL, IQR 219.5 - 319.5, p <0.05) was found to be the independent risk factor. Patient initiating ART when CD4 count was >350 CD4 T-cell count (n=130) comprised more INR, followed by CD4 count of 200 – 350 cells, then <200 cells (p value <0.05).

Conclusion: Despite the suppressed viral load, significant proportion of patients found to have low CD4 recovery and associated with high baseline /nadir CD4 count and lower baseline viral load. Understanding the pathophysioligic mechanisms responsible for this immune disconnect could be explored in clinical practice for the most effective management of discordant patients to improve the clinical outcome.
Prevalence of genital herpes in HIV positive patients attending STI clinic at a tertiary care hospital and its correlation with CD4 counts

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Background: HIV infection is emerging as one of the major health problems faced by the clinicians across the world, more so because of co-existence of many sexually transmitted infections (STIs) among HIV infected patients. The presence of untreated STIs (both ulcerative and non-ulcerative) increases the risk of both acquisition and transmission of HIV by a factor of up to 10 times. Recurrent and persistent ulcerative HSV2 lesions are among the common infections in HIV patients. Prevalence of genital herpes has increased markedly between the 1970's and 1990's. Hence it is important to promptly diagnose and treat genital herpes which thus concurrently reduces the transmission of HIV.

Methods & Materials: A detailed history of 200 retropositive patients with regard to name, age, sex, nature and duration of illness was noted. Photographs of the lesions were taken for documentation. Diagnosis of herpes genitalis was done mainly on clinical grounds Tzanck smear and HSV2 IgM antibody titers were done. To rule out other causes of genital ulceration, samples were sent for appropriate microbiological investigations. CD4 T cell counts were done. The results were tabulated and appropriate statistical tests were done

Results: Out of 200 retropositive patients 52 (25.5%) presented with STIs among which 30 had genital herpes (15%). It was more commonly seen in the age group of 30-40 years, married couples and patients having multiple partners. Tzanck smear and IgM anti HSV2 positivity was seen in 43% and 48% of patients respectively. Average CD4 count of 297 cell/cu.mm was seen.

Conclusion: Trend of STI's has gradually changed over the years, with decline in incidence of bacterial STI's & increase in the prevalence of viral STI's most commonly genital herpes. HIV and herpes genitalis coinfection increases the transmission of each other.
Plasma cytokine and chemokine levels and their impact on HIV disease non-progression among HIV-1 subtype-C long-term non-progressors from South India

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Background: Plasma cytokine and chemokine levels in HIV infected individuals are known to have direct association in disease progression. However, this association is not well elucidated between different groups of HIV infected individuals. Immune control mechanisms at cellular level are often attributed to disease non-progression in long-term non-progressors (LTNPs). Hence, to investigate the possible association of plasma cytokine and chemokine levels with disease progression, in this preliminary study we have evaluated the relative proportion of cytokine and chemokine levels in plasma among LTNPs and progressors. We also correlated the levels with the markers of disease progression such as CD4 T-cell count and plasma viral load (PVL).

Methods & Materials: Concentration of cytokines - IL-2, IFN-γ, TNF-α, IL-6 and IL-22 and chemokines - IP-10, MIP-1β, MCP-1 and RANTES in plasma were estimated using ELISA in LTNPs (n=15) and progressors (n=13). Statistical analyses were performed using Mann Whitney U test and Spearman rank correlation test. A p value of <0.05 was considered significant.

Results: The median age of LTNPs were 38 years with median CD4 T-cell count of 870 cells/µL and median PVL of 154 copies/mL, while the median age of Progressors were 30 years, with median CD4 of 384 cells/µL and median PVL of 1,39,308 copies/mL. Opportunistic infections such as tuberculosis (TB), and gastrointestinal infections were reported in 9 of 13 progressors (69%). IFN-γ (p=0.0009) and IP-10 (p=0.018) levels were significantly elevated in progressors than in LTNPs, whereas MCP-1 (p<0.005) and RANTES (p=0.03) were significantly increased in LTNPs. No significant correlation was found between disease progression markers and the levels of cytokines and chemokines.

Conclusion: Unlike observed in cellular expression, plasma levels of CXCR3 ligand, IFN-γ were lower in LTNPs, which in turn might have influenced the lower IP-10 levels in LTNPs. Also, higher MCP-1 and RANTES levels in LTNPs might have resulted in higher monocyte infiltration and CCR5 receptor binding. Minimal events of opportunistic infections, lesser migration of TH1 and TH2 cells due to lower cytokine activity at peripheral circulation and higher inhibition of HIV binding to co-receptors due to elevated chemokine levels might explain the possible reasons behind disease non-progression in LTNPs.
Micro-level social and structural syndemic of HIV risk among Nepalese female sex workers

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**Background:** Sex workers face stigma, discrimination and violence across the globe and are almost 14 times more likely to be HIV infected than other women in low-and middle income countries. In Asia, condom campaigns at brothels have been effective in some settings, but for preventive interventions to be sustainable it is important to understand micro-level social and structural factors that enable sex workers to practice safer sexual behaviors. This study assesses the syndemic effects of micro-level social and structural factors of unprotected sex and the prevalence of HIV among female sex workers (FSW) in Nepal.

**Methods & Materials:** In this quantitative study, 610 FSW were recruited using two-stage cluster sampling between September 2012 and November 2012 from 22 Terai highway districts of Nepal. Rapid HIV tests and face-to-face interviews were conducted to collect biological and behavioral information. A count of physical (sexual violence), social (poor social support and condom negotiation skills) and economic (unsafe sex to make more money) factors that operate at the micro-level was calculated to test the additive relationship to unprotected sex. Unprotected sex was assessed with the following question: “The last time you had sex with your client, did he use a condom?” Point-biserial correlation was conducted to measure the size and significance of associations between each syndemic condition and unprotected sex. Statistically significant associations between independent variables and unprotected sex were computed using multivariable logistic regression.

**Results:** The HIV prevalence was 1% in this presumably representative and large sample of FSW in Nepal. The prevalence of unprotected sex with client was high (24%). For each additional adverse physical, social and economic condition, the likelihood of non-use of condoms with clients increased substantially: 1 problem=2.2 odds ratio (OR); 95% confidence interval (CI)=1.3-3.7; 2 problems=3.1 OR; 95% CI=1.8-5.4; 3-5 problems=7.3 OR; 95% CI=3.9-13.9.

**Conclusion:** Interactions between two or more adverse conditions linked to physical, social and economic environment increased the risk of unprotected sex among FSW. A more holistic approach, including efforts to improve condom negotiation skills and to address economic vulnerability and abuse, is required to address unprotected sex among FSW in Nepal.
Poor condom-negotiation skills, inadequate social support, depression and incarceration associated with HIV risks among young key populations in Nepal
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Background: Female sex workers (FSW), people who inject drugs (PWID) and men who have sex with men and transgender (MSM/TG) are more at risk to be infected with HIV, especially if young. This study assess the HIV prevalence and examines the effects of physical, social, economic and policy factors—that operate at the micro and macro level—on risk behaviours among young key populations in Nepal.

Methods & Materials: Out of a total of 721 young respondents (16-24 years), 215 FSW and 308 PWID were recruited through two-stage cluster sampling, while 198 MSM/TG were recruited through respondent driven sampling (RDS) technique. RDS related estimates were calculated using Giles SS estimator. Logistic regression identified correlates of non-use of condoms and use of unsafe needles and syringes.

Results: The prevalence of HIV among PWID, FSW and MSM/TG was 3%, 2% and 1%, respectively. Risk behaviours in the different groups ranged between 13-22%. A high level of depression was found in all the three key populations (between 8-51%). Unable to negotiate condom use and poor social support were associated with non-use of condoms among FSW. Late sexual debut for money and depression were associated with non-use of condoms among MSM and TG. Injection frequency, poor social support and incarceration were identified as barriers in reducing unsafe needles and syringes use among PWID.

Conclusion: Factors operated in both the micro and macro levels are influencing HIV risk behaviours among young key populations in Nepal. Future prevention strategies need to address these factors and target those young key populations who are most vulnerable.
Ecological study of HIV cases with socio-crime factors associated in Indonesia

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**Background:** Previous publication presented in International AIDS Conference in Vienna, 2010, shows that women who experience domestic violence have potential risk of contracting HIV at least two times greater. WTO report in 2013 also support the fact that in some regions, women who are victims of domestic violence are 1.5 times more likely to contract HIV than those who do not have experienced violence with their intimate partner. Corruption is considered factors if there is misappropriation funds that hampering the HIV prevention program in the country. Indonesia has Corruption Perception Index 107/175 in the world. Until present, the HIV case and its socio-crime factors in Indonesia has not been fully understood. The study aim to measure association of domestic violence and corruption with HIV cases in Indonesia.

**Methods & Materials:** Aggregated data of HIV cumulative cases and criminal statistics per-province are collected from National statistics bureau, 2013. Pure ecological study is conducted using cross-sectional design. Two socio-crime factors: domestic violence and corruption are determined as the independent variables. **Results:** The analysis shows that HIV cases per-province in average $e^{1.367982}$ or 1584. Poisson regression estimate for one province increase in HIV cases, given the other variables are constant. If a province were to increase the each independent variable by one point, the incidence rate for HIV cases would be expected to increase by factor $1.002075$ of domestic violence (CI 95% 1.002046-1.002105, $P<0.001$) and $1.057$ of corruption (CI 95% 1.056-1.058, $P<0.001$).

**Conclusion:** This indicates that domestic violence and corruption is associated with the increased of HIV cases in Indonesia. However this study can contain ecological fallacy because the global measurement by province is used. It is recommended to have further study using individual data. The study will be benefit to improve the HIV prevention program in Indonesia.
Archived drug resistance profile among suppressed HIV patients using conventional and sensitive allele specific PCR in Tenofovir experienced patients in South India


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Background: Drug resistance (DR) is one of the major hurdles in HIV treatment. HIV RNA based DR testing is relatively expensive and infeasible compared to DNA based, in setting where transport of specimen to regional/central laboratory is needed. Previous report states concordance between RNA and DNA DR pattern. Patient with suppressed viral load (SVL) may harbor DR, which cannot be detected by conventional DR assay; hence PBMC DNA sequencing and Allele specific PCR (ASPCR) which can quantify minority variants, can predict adherence status and predisposition to failing treatment; which can be managed earlier.

Methods & Materials: We examined reverse transcriptase (RT) sequence in PBMC DNA of patients with SVL for DR using conventional Sanger sequencing and ASPCR for RT mutations K103N and K65R. DR was based in 2014 IAS-USA DR list.

Results: We analyzed n=90 on TDF regimen (82, 1st line and 8, 2nd line), with median age 38 years, n=50 being female; all had subtype C infection and showed monophyletic clustering. Any DR mutations (DRM) were observed in n=12 (13.3%), NRTI DRM in n=8 (8.9%), NNRTI DRM in n=11 (12.2%), and both in n=8 (8.9%). Major NRTI and NNRTI DRM observed was M184I/V (7.8%), K70R (3.3%) and V106A/M (4.4%), K103N (3.3%) respectively. Although, population genotyping shows K65R and K103N DRM in none and 3.3%, respectively. ASPCR result shows 32/30 had >1%, 8/13 had > 5% and 3/9 had >10% K65R/K103N, respectively. K65R, and K103N negative by population genotyping and positive (>10%) by ASPCR was observed in 3.3% and 6.7%, respectively. Mutation pattern shows none showed resistance to TDF despite being in that regimen, in contrast resistance for NVP and EFV was observed in 7.8%.

Conclusion: PBMC genotyping among suppressed has shown resistance mutation among 13%, does the mutations observed reflect recent archival is a big question. In this scenario how reliable it is to manage HIV infection among suppressed taking archived DR into consideration, needs to be substantiated. In the other way on considering PBMC DNA as recent archival; based on ASPCR result, the minor proportion of resistant virus corroborates the ongoing minimal viral replication, which is one of the hurdle in HIV cure.
Low virulence of HIV-1 subtype C underlies treatment success despite high baseline viral loads
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Background: High baseline viral load is implicated in rapid disease progression, as is often the case in resource-limited settings. Intriguingly, despite higher baseline viral loads, first-line antiretroviral treatment (ART) of HIV-1C infection in India elicits a response comparable to that of HIV-1B infection in the west. Models of viral dynamics, applied to HIV-1B infection, have shed light on viral decay dynamics and elucidated markers of treatment outcome, although such studies for HIV-1C are lacking. We hypothesized that the HIV-1C strain in India is less virulent than the HIV-1B strain in the west, leading to a favourable treatment response.

Methods & Materials: To test this hypothesis, we measured viral decay dynamics during treatment and analyzed the data using a mathematical model to estimate the within-host basic reproductive ratio, \( R_0 \), a quantitative measure of virulence, and the critical efficacy for successful treatment, \( e_c \). Patients were initiated on first-line ART in India and followed for the first 6 months of treatment. Viral load, CD4\(^+\) T-cell count, and adherence data were collected at baseline, 4, 12, 16 and 24 weeks following ART initiation. Drug resistance genotyping was done at baseline.

Results: Among 257 patients with complete data (mean age 36.2 years and 60% male), mean baseline viral load was 5.7 log\(_{10}\) copies/mL. At 6 months, 87.5% had undetectable viral load. Sub-optimal adherence (<95%) (p<0.001) and primary drug resistance mutations (p=0.029) were associated with virological non-response. Our mathematical model, considering the dynamics of productively and long-lived infected cells, provided good fits to the viral load data. We estimated the median \( R_0 \) to be 5.3 (IQR: 4.5-7.1), which is significantly smaller (p=0.001) than current estimates for HIV-1B (median \( R_0 \approx 8 \)), indicating lower virulence of HIV-1C than HIV-1B. The corresponding \( e_c \) for HIV-1C is \( \approx 0.8 \), again smaller than that for HIV-1B.

Conclusion: The lower \( R_0 \) and \( e_c \) imply that responses can be achieved with lower dosages and/or adherence, potentially explaining the favourable treatment response of HIV-1C infection in India despite the high baseline viral loads. The lower virulence of HIV-1C may also underlie its growing global spread.
HLA-C*07 allele group confers protection against cytomegalovirus retinitis development among Brazilian AIDS patients

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Background: Even though antiretroviral therapy has reduced the incidence of cytomegalovirus retinitis (R-CMV), this disorder continues to be a considerable cause of visual impairment among AIDS patients. The incidence of R-CMV is increasing in young patients outside the first world who, despite the control of HIV-1 viremia, are blind, either because retinitis has not been diagnosed or has incorrectly been treated. An insertion/deletion fragment at the 3' untranslated region of the HLA-C gene has been associated with progression to AIDS after HIV infection. Thus, the search for genetic markers associated with disease susceptibility/morbidity may help to identify AIDS patients prone to develop R-CMV.

Methods & Materials: Four groups of patients were typed for HLA-C: GI (n=52), consisting of patients with AIDS and R-CMV; GII (n=170) patients with AIDS without R-CMV; GIII (n=222) encompassing GI and GII; and GIV (n=202), healthy HIV- individuals. HLA-C typing was performed using commercial kits. The allele frequencies were compared between groups and the etiologic and preventive fractions were calculated. Intergroup differences were analyzed by the Fisher exact test and the odds ratio (OR) and 95% confidence interval (95%CI) were calculated, with the level of significance set at \( P \leq 0.05 \).

Results: The HLA-C*07 allele group was underrepresented in AIDS patients with R-CMV (OR: 0.4192, 95% CI 0.2246–0.7822, \( P=0.0054 \)) when compared to controls or when compared to AIDS patients without R-CMV (OR: 0.4233, 95% CI 0.2246–0.9782, \( P=0.0063 \)), conferring preventive fraction of 0.1553 and 0.1531, respectively.

Conclusion: The HLA-C*07 allele group was associated with protection against R-CMV, and the study of HLA-C*07 haplotypes encompassing the coding and 3'UTR segments may help on discriminating AIDS patients prone to develop R-CMV.

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**Background:** As of 2012, 3,400 000 million people (all ages) are living with HIV in Nigeria. The estimated new HIV infections is 260,000 and estimated AIDS death is 240,000. The reported number of adults on ART(Anti-retroviral treatment) was 459,465 and the ART coverage based on WHO guideline was 36%. The number of pregnant women living with HIV who received antiretroviral for preventing mother-to-child transmission was 33,323 and the percentage coverage was 17%. Enugu State has the highest prevalence (6.5%) of HIV/AIDS in the South East and the fourth in Nigeria. To implement the commitments in the 2011 United Nations Political Declaration on HIV and AIDS and increase progress towards universal access to HIV prevention, treatment, care and support, Nigeria has developed the president’s Comprehensive Response Plan (PCRP). We determined the implementation of these preventive services by health care providers in Enugu State.

**Methods & Materials:** I reviewed 2010-2013 HIV/AIDS Surveillance data of Enugu State. We conducted descriptive analysis of ART utilization, PMTCT and HCT services using Microsoft Excel 2007.

**Results:** The total number of all individuals that accessed HCT services from 2010 to 2013 was 87,000, 104,344, 161,517, and 113,903 respectively. The total number of HIV positive individuals from 2010 to 2013 was 8,965(10.3%), 7,695(7.3%), 9,233(5.7%), and 6,110(5.4%). respectively. The overall total number of individual newly started on ART from 2010 to 2013 was 15,629. The percentage of pregnant women counseled, tested with result was 23,100, 41,000, 45,318, and 38,440 respectively for 2010-2013 and those that tested positive are for 2010-2013 are 1,059(4.6%), 1,363(3.3%), 1,845(4.1%), and 1,036(2.7%). Among the pregnant women that tested positive, the number that are receiving ART for 2010-2013 are 1,028(92.0%), 1,041(76.4%), 1,640(88.9%), and 719(69.4%) respectively

**Conclusion:** The state AIDS and STI control Programme, though has achieved success in the prevention of HIV/AIDS as evidenced by decreased percentage of HIV/AIDS positive individuals over the years under study and decreased percentage of newly diagnosed HIV positive cases among pregnant women, the State still need to scale up the ART coverage among pregnant women by increasing the number of facilities that renders ART services in the state.
HIV missed ...CMV shows the way

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**Background:** We present a case of transplacentally acquired human immunodeficiency virus (HIV) infection in a child who presented with disseminated cytomegalovirus (CMV) infection.

**Methods & Materials:** We present a case of transplacentally acquired human immunodeficiency virus (HIV) infection in a child who presented with disseminated cytomegalovirus (CMV) infection. A 16 months old female child presented with complaints of failure to thrive, regression of developmental milestones and 2 episodes of convulsions since 10 months of age. Child was well till age of 10 month followed which started showing regression of milestones like walking with support, standing and sitting without support, speaking bisyllables which she was able to do before. Child had signs of severe acute protein energy malnutrition like dry thin brittle hair, dry skin. Examination also revealed microcephaly, icterus, oral thrush, no eruption of primary teeth and hepatomegaly.

**Results:** Complete blood count showed severe anaemia (4.7gm) and thrombocytopenia (78000) and leucocytosis (18400). Renal function tests (Sr. creatinine= 2.1, blood urea=71) and liver function tests (SGOT =1231, SGPT= 258 serum bilirubin=4.8) were found to be deranged. Opthalmoscopic examination was strongly suggestive of Cytomegalovirus retinitis. Infection was confirmed by a positive result for CMV on polymerase-chain-reaction analysis of blood and urine. With the clinical picture of disseminated CMV infection, immunodeficiency was strongly suspected. HIV DNA PCR of both child and mother was positive for HIV-1. Absolute CD4 count of child was 298 and percentage was 33%. To combat active retinitis, intravenous ganciclovir was started and planned to start antiretroviral treatment 2-3weeks later.

**Conclusion:** Early detection and prompt treatment of HIV and associated opportunistic infections is of utmost importance. Paediatric HIV/AIDS can present as failure to thrive and regression of acquired milestones. Initiation of highly active antiretroviral therapy should be done after adequate control of underlying opportunistic infections to prevent immune reconstitution inflammatory syndrome.
Background: India with 2.1 million HIV populations, good access to first-line antiretroviral therapy is widely available, however understanding HIV Transmitted Drug Resistance (TDR) and polymorphism is critical for continued success.

Methods & Materials: HIV-1 pol gene spanning 20-240 codons of RT was genotyped by validated homebrew method for 100 ART naïve participants. The sequences were analyzed according to WHO recommendations using the Calibrated Population Resistance (CPR) tool of Stanford University HIV drug resistance (DR) database. For polymorphism identification subtype C consensus was used. Sequence was aligned (Clustal X) to an Indian subtype C reference C.IN.AF067155) and examined for HIV-1 subtype using REGA V3. Majority were subtype C infected 99% (n=99).

Results: Among 100, 61 were male, with median age and CD4 count of 37 years (IQR 27-38), and 244 cells/uL (153-359) respectively. TDR was observed among 4 participants, which is considered to be low level based on the WHO recommendations for public health action in response to TDR survey results. Observed mutations were M41L (1%), K219R (1%), K101E (1%) and Y181C (1%). The observed NRTI DRMs has less effect on available NRTI options while NNRTI DRMs will have adverse impression on available NNRTI options. Apart from TDR, naturally occurring polymorphism is also a main concern as some polymorphism may cause resistance to ART (eg. V90I, V118I, E138A, V179D). Based on that all the patients had polymorphic mutations and the predominant polymorphisms observed were A36E (41%), K211R (36%), A162S (35%), in addition polymorphic mutation causing resistance such as V90I, V118E, E138A and V179D was seen in 2, 1 and 3 individuals respectively. If subtype B consensus was taken for polymorphism identification, positions of RT such as 35, 36, 39, 48, 60, 121, 122, 162, 173, 177, 200, 207 and 211 would be overestimated as &gt;60% polymorphism in that position.

Conclusion: We conclude, low prevalence of HIV DR among naïve participants. In addition 17 positions known to be polymorphic in subtype B (between 20-240 codons) has different amino acid consensus in subtype C. Thus polymorphisms pertaining to subtype C should be functionally annotated for DR.
Low plasma nevirapine levels during antiretroviral treatment initiation and dose escalation in HIV-infected children: therapeutic implications

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Background: Nevirapine, a component of antiretroviral therapy (ART) in resource-limited settings is known for auto-induction of metabolism, and thus is prescribed at half therapeutic dose until day 14 (‘lead-in period’), and then escalated to full dose. However, young children have higher clearance rates, suggesting that dosing strategy based on adult studies may not be appropriate in children. We aimed to determine plasma nevirapine concentrations achieved during the first month among children initiated on nevirapine-containing ART

Methods & Materials: ART-naïve HIV-infected children, initiating ART were included in this prospective study. Plasma nevirapine trough levels were analyzed in duplicate (with additional 10% repeats for quality control) by high performance liquid chromatography (HPLC) (lowest detection limit 0.062 µg/ml) at days 7, 14 (lead-in period) and 28 (full dose) after ART initiation. Baseline transmitted drug resistance genotyping, serial CD4 count and viral load was done.

Results: Among 28 children aged 2-12 years initiated on nevirapine-based ART between 2013-2014, six were excluded due to drug toxicity, and two were transferred out within first month. Among 20 children included in the study, 19 reported >95% adherence at all study visits. Transmitted drug resistance was seen in 5(10%) children (V90I, K103N, K101E, E138G, Y188L, M41L, L210W, T215Y). Median trough nevirapine concentration was 4.83µg/ml (IQR 3.48-6.06) at day 7 of ART initiation, 3.35µg/ml (IQR 2.06-7.91) at day 14 (lead-in period), and 7.97µg/ml (IQR 5.55-10.66) on day 28 (p= 0.018). During the lead-in period 40% (8/20; 15% at day 7, 35% at day 14) of children failed to achieve therapeutic levels of >3 µg/ml, while after dose escalation, only 5% (1/20) had low therapeutic levels. Low trough nevirapine levels was not significantly associated with age, viral load, CD4 count or transmitted drug resistance.

Conclusion: Our results showed significantly lower therapeutic concentrations of nevirapine during the lead-in period in young children initiating nevirapine-based ART in India. Given nevirapine's low genetic barrier, sub-therapeutic levels during the early high viremic period can lead to later drug resistance development and treatment failure. Long-term drug resistance studies among viral quasispecies can determine the true effect of early subtherapeutic concentrations.
Knowledge and practice of the prevention of mother-to-child transmission of HIV guidelines among doctors and nurses at Tswane District, South Africa

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Background: Since the beginning of the HIV/AIDS epidemic, almost 60 million people have been infected and 25 million people have already died from HIV related causes, (WHO, 2010). Sub Saharan Africa is the region most affected with 67% of all people living with HIV worldwide and 91% of all new infections among children. In South Africa, the mother-to-child HIV transmission (MTCT) rate is under 4% at 4 to 8 weeks after birth since the implementation of the most recent national PMTCT programme. The PMTCT programme in South Africa has gained rapid momentum in training and practice.

Methods & Materials: This study was conducted in Gauteng province of South Africa. We sought to investigate the level of knowledge and whether the doctors and nurses working at Odi were practicing the current PMTCT program. A descriptive cross sectional survey using self-administered questionnaires was used. The study population consisted of 31 doctors and 180 nurses.

Results: There were 102 participants. There were 12 (12%) doctors and 90 (88%) professional nurses. There 9 (9%) males and 93 (91%) females. Thirty four participants (33%) underwent further training beyond their undergraduate qualification. The mean knowledge percentage per participant was 60.8% and 77% for the mean practice percentage per participant. In the knowledge questions, the question on the first step (HIV counseling and testing) scored an average 93.1% (highest) by participants and the doses of drugs used in the PMTCT guidelines scored 17.7% (lowest) by participants. In the practice questions, the score for each question ranged from 71% to 82%.

Conclusion: Nurses and doctors working at Odi Hospital know that HIV counseling and testing is important and must be done on all mothers; however they were unsure of the dosages of the drugs used for PMTCT. More than two thirds of the doctors and nurses reported to be practicing the PMTCT guidelines, however as their knowledge was inadequate, their enthusiasm is lost by actually not knowing the correct drugs and dosages for the PMTCT which impacts on the correct PMTCT practice.
The characteristics of HIV/AIDS patients with deep vein thrombosis at Dr George Mukhari Academic Hospital

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**Background:** Deep vein thrombosis (DVT) is ten times more prevalent in HIV/AIDS patients than the general population. HIV/AIDS has also been shown to be a hypercoagulable state which is worsened by conditions like malignancies, opportunistic infections, some auto-immune diseases and chemotherapeutic agents.

**Methods & Materials:** This was a cross sectional, descriptive study looking at all HIV/AIDS patients admitted to level one wards at the hospital without DVT.

<table>
<thead>
<tr>
<th>Results: Variable</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>On HAART</td>
<td>6</td>
<td>35%</td>
</tr>
<tr>
<td>On Tuberculosis treatment</td>
<td>8</td>
<td>47%</td>
</tr>
<tr>
<td>Improvement of DVT</td>
<td>16</td>
<td>94%</td>
</tr>
<tr>
<td>Pneumonia excluding Tuberculosis</td>
<td>4</td>
<td>24%</td>
</tr>
<tr>
<td>Discharged on warfarin</td>
<td>17</td>
<td>100%</td>
</tr>
<tr>
<td>Attempted suicide</td>
<td>2</td>
<td>12%</td>
</tr>
<tr>
<td>Cerebro vascular accident</td>
<td>1</td>
<td>6%</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>4</td>
<td>24%</td>
</tr>
<tr>
<td>Anaemia – recorded</td>
<td>2</td>
<td>12%</td>
</tr>
<tr>
<td>Mean duration of admission</td>
<td>14.1 days</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:** Patients with HIV/AIDS and opportunistic infections, or other predisposing factors such as immobility are more likely to develop DVTs.
The profiles of HIV-infected patients treated at A. Wahab Sjahranie General Hospital Samarinda, Indonesia

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**Background:** The number of HIV-infected patients and deaths-related to HIV are increasing rapidly in Indonesia. Studies about the profiles of HIV-infected patients can be useful to create better approaches for prevention and treatment.

The aim of this study was to evaluate the profiles of HIV-infected patients who got ARV treatment in VCT clinic at A. Wahab Sjahranie General Hospital Samarinda.

**Methods & Materials:** This study was conducted at A. Wahab Sjahranie General Hospital in Samarinda, East Kalimantan, Indonesia from December 2005 to September 2015. Subjects were HIV-infected patients who got ARV treatment at VCT clinic.

**Results:** Until September 2015 there were 394 patients who had taken ARV, 96 (24.4 %) of them had died. Among 298 patients who were on ARV treatment, there were 172 males (57.7 %) and 126 females (42.3 %) with ages distribution were as follow: ≤ 10 years 10 (3.4 %), 11-20 years 11 (3.7 %), 21-30 years 118 (39.6 %), 31-40 years 104 (34.9 %), 41-50 years 36 (12.0 %), > 50 years 19 (6.4 %). Routes of transmission were as follow: sexual 274 (91.9 %), IDU 10 (3.4 %), mother to child 10 (3.4 %), others 4 (1.3 %). CD4 levels when starting ARV: < 50 : 107 (35.9 %), 51-100 : 59 (19.8 %), 101-200 : 69 (23.2 %), 201-350 : 43 (14.4 %), 351-500 : 14 (4.7 %), > 500 : 6 (2.0 %). The most common ARV regimen used for adults was TDF-3TC-EFV (71.8 %). Patients who started taking ARV when pregnant were 19 (6.4 %). Adherence to ARV: > 95 % 227 (76.2 %), 80-95 % 23 (7.7 %), < 80 % 48 (16.1 %).

**Conclusion:** The majority of patients who get ARV treatment at VCT clinic of A. Wahab Sjahranie General Hospital Samarinda are young people (21-40 years old), and the major route of transmission is sexual intercourse. Most of the patients start taking ARV in a late phase of infection which is correlated with poor prognosis.
Metabolic syndrome among people living with HIV (PLHIV)

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Background: India ranks third in the world for number of people living with HIV (PLHIV). Anti-Retroviral Therapy (ART) has led to decline of morbidity and mortality, making HIV a chronic manageable disease. This increased life span extends exposure to environmental/lifestyle risk factors contributing cardiovascular diseases (CVD) and other diseases of aging. Metabolic syndrome (MS) is a clustering of risk factors for CVD, and an important public health concern. Globally prevalence of MS among PLHIV varies from 11 – 45%, but data are sparse on MS among PLHIV in India. We describe the prevalence of MS among PLHIV population from southern India.

Methods & Materials: A cross-sectional study with non-probability consecutive sampling in a private clinic of Hyderabad was carried out. Adult PLHIV under the care of the physician consultant, with informed consent, were included in the study irrespective of their ART status. Structured questionnaire, anthropometric measurement and fasting blood samples were drawn. Metabolic syndrome was defined using International Diabetes Federation (IDF) and U.S. National Cholesterol Education Program Adult Treatment Panel III (ATPIII) criteria, with specific cut offs for Asian Indians. Highly sensitive C Reactive Protein (Hs-CRP), a marker of inflammation and of CVD risk, was also measured. The study aims at recruiting 400 PLHIV; we present the preliminary findings of 225 participants.

Results: There were 63 % males. Mean age was 43 years, 7.5 years mean duration since HIV diagnosis, and 97% initiated on ART. Based on the ATPIII, 52% (57% Males & 42% Females) and IDF 46% (49% Males & 41 Females) met the criteria for MS. Age was higher among PLHIV with MS by either criteria. 40% had >3 mg/dl Hs-CRP levels, with males (median 2.37, Q1 1.1, Q3 4.25) and females (median 2.31, Q1 1.2, Q3 4.97) similar statistically. The Hs-CRP levels were not associated with MS categorized by ATP III or IDF criteria.

Conclusion: The overall prevalence of MS and the Hs-CRP levels were high in this PLHIV population. With the diverse and inconsistent evidence globally on the CVD risk associated with MS and Hs-CRP among PLHIV, further research efforts are required to delineate this dual burden among PLHIV.
Retrospective analyses of CD4 count monitoring to detect ART response

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Background: National AIDS Control Programme was launched in 1992 by National AIDS Control Organization for prevention and control of HIV/AIDS in India. Viral load testing is a preferred test to monitor treatment response of antiretroviral therapy (ART). However, for more than two decades, CD4 cell count measurements have been used as a major marker in understanding HIV disease progression, making important clinical decisions, and monitoring the response to ART. In India, most of the laboratories have been using CD4 count for monitoring the response of ART. The study is undertaken because there is limited data available about response to ART.

Methods & Materials: HIV cases who presented at the ART clinic of JIPMER and were on ART from September 2014 to August 2015 were included in the retrospective study. Patient demographic details and CD4 count were recorded. A patient on ART was considered non-responsive to the ART if the CD4 count didn’t rise after six month of ART regime.

Results: A total of 581 HIV positive patients presented at the ART clinic of JIPMER during the study period. 300 (51.6%) were male and 281 (48.4%) were female. 448 patients (77%) were resident of Tamil Nadu and 133 (23%) were from Pondicherry. 442 patients (76%) were on ART and CD4 count were recorded. CD4 count of 126 (28.5%) patients didn’t rise from their previous CD4 count and were considered non-responsive to the ART. 69 (54.8%) were female and 57 (45.2%) were male.

Conclusion: In resource limited settings CD4 count testing is most commonly done to monitor the ART response in HIV positive patients. It would detect treatment failure and support clinical decision for starting the patient on second line ART. 28.5% of patients on ART didn’t observe improvement in their CD4 count. These cases were observed more in females (54.8%) than males (45.2%). It may be due to non-compliance of ART by these patients. There is a risk of development of HIV drug resistance in these cases. Large scale similar study will be required to generate broader data which will facilitate better ART care.
Association of nadir CD4 counts with carotid-intima media thickness and inflammation markers in HIV infected patients

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**Background:** During HIV infection, apart from use of PI-based regimen, low CD4+ T-cell count has also been identified as a vascular risk factor. Initiating ART in patients with higher nadir CD4 counts is speculated to better normalise CVD risks and inflammatory response. We aimed to study effect of nadir CD4 count on CVD and inflammatory response in ART naive and treated HIV patients.

**Methods & Materials:** Cross-sectional enrolment of 169 HIV-infected patients (68 naive; 101 ART experienced) with different nadir CD4 counts; Drug Naive (DN)- Group 1 (n=24; Nadir CD4<350cells/µL); Group 2 (n=44; Nadir CD4>350cells/µL); ART experienced- Group 3 (n=32; Nadir CD4 <200cells/µL); Group 4 (n=36; Nadir CD4 200-350cells/µL); Group 5 (n=33; Nadir CD4 >350cells/µL) and Group 6 (n=29) healthy controls (HC) were done. We measured serum lipid profile (LP), C-IMT, cardiac output, TNFR-1, TNFR-2 for inflammation, sCD14 for microbial translocation (MT) by ELISA. Descriptive statistics were used for demographics; ANOVA to identify differences in LP, C-IMT, cardiac output, inflammation, MT between groups.

**Results:** Mean age, median current and nadir CD4 count for groups 1-5: 37.7±5.6, 123.5(70-273.5), 123.5(70-273.5); 35.36±5.2, 534(440-595), 572(496.5-761.25); 38.7±5.7, 138.5(83-167.5), 569(430.25-773.5); 38.3±5.9, 273.5(228.5-317.5), 717(564-867.5); 40.3±5.1, 402(378-438), 777(649-1005); 37±5.9. C-IMT (p<0.05), TC, TG, TNFR-2, sCD14 (p<0.05) increased in DN and ART than HC. Cardiac output (p<0.001) decreased, TNFR-1 (p=NS), TNFR-2 (p<0.001), sCD14 (p<0.001) higher in DN than ART. No significant difference in C-IMT between groups based on nadir CD4 counts. In naive patients, increased cardiac output (p<0.001), decreased TNFR-1 (p<0.001), TNFR-2 (p<0.001) and sCD14 (p<0.001) was seen in group 2 than group 1. We did not identify statistically significant difference in LP, cardiac output, TNFR-1, TNFR-2, sCD14 levels between groups 3-5. We identified significant correlation (p<0.05) between low CD4 count and increase in C-IMT on ART patients.

**Conclusion:** From clinical perspective, no significant betterment in terms of decrease in inflammatory response, C-IMT and increase in cardiac output was identified during early initiation of ART. However, we found significant increase in inflammatory and MT markers in naive patients with nadir CD4 <350 cells/µL than those with higher nadir CD4 count.
Anxiety levels of HIV-infected patients after learning their diagnosis: A preliminary study for the first time in Turkey

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Background: HIV infection is not well known in population, and knowledge of HIV contains many myths. Fear of unknown diagnosis may ease increasing of anxiety feelings. In this study we aimed to search spontaneous anxiety levels of HIV-infected patients when they learn the diagnosis.

Methods & Materials: The study was conducted by the departments of psychiatry and microbiology in Istanbul University, Cerrahpaşa Faculty of Medicine. Semi-structured data form and STAI –I and II scales was performed to the patients after explaining their replicated HIV (+) state.

Results: 39 male and 2 female were included in this study. Mean age of study group was 34,9. Graduation of the participants were sequentially, 58,5 % (n:24) university, 14,6 % (n:6) high school, and 26,8% (n:11) primary school. 82,9% of the group was actively working. Mean age of HIV (+) state was 30,1. Sexual transmission (70,7%, n: 29) was major infection resource of HIV. Partner disclosure rate of group was 46,3% (n: 19). Mean STAI-I state anxiety level was 56,1, and mean STAI-II trait anxiety score was 43,6.

Conclusion: Although the results of our preliminary study revealed high anxiety levels of patients after learning their HIV (+) state, after all of the cases were completed, we may come to a more definite conclusion. According to our preliminary results, high anxiety levels of HIV-infected patients may be related with high stigma of society over HIV-infected people and lack of knowledge about the infection.
Caregiver burden among adults caring for people living with HIV/AIDS (PLWHA) in South India
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**Background:** Caregiver burden refers to the physical, emotional and financial hardships associated with caregiving for an ailing individual. Attending to the needs of people living with HIV/AIDS (PLWHA) can place a significant burden on family members. This may adversely affect their quality of life (QOL). The main aim of our study was to assess the caregiver burden and QOL among family members of PLWHA in South India. We also tried to determine the impact of caregiver burden on QOL.

**Methods & Materials:** This cross-sectional hospital-based study was carried out at Kasturba Medical College (KMC) Mangalore. The study was conducted over a period of eighteen months starting from October 2013. A total of 360 caregivers voluntarily participated in our study. The data were collected by face-to-face interview. Caregiver burden was assessed using the Zarit Burden Scale. WHOQOL-BREF Questionnaire was used to assess the QOL of caregivers. The collected data were entered and analyzed using SPSS version 16.0. The protocol was approved by the Institutional Ethics Committee.

**Results:** The mean age of caregivers was 36.09±10.18 years. Most of the caregivers were females (77.5%). Majority of caregivers (51.1%) belonged to Middle/Lower Middle socioeconomic class (Kuppuswamy class III). In our study, 36 (10%) caregivers had very severe burden and 88 (24.4%) had moderate to severe burden. Physical domain of QOL showed maximum score of 60.28±13.08, while a minimum score of 51.88±14.20 was seen in social domain. With increase in caregiver burden the mean QOL scores decreased which was statistically significant.

**Conclusion:** Our study highlights the need to counsel the caregivers on how to deal with PLWHA in the family. Family care plays a major role in the general well-being of PLWHA. Majority of national HIV programmes all over the world focus mainly on PLWHA. National programmes should immediately address the mental health issues of caregivers thereby reducing caregiver burden. More studies on this topic have to be conducted in developing countries.
HIV-mediated CD8 encephalitis: An under recognised entity

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Background: Combined antiretroviral therapy (cART) has been associated with significant decrease in the mortality and morbidity in patients with HIV/AIDS. However, there has been increased recognition of immune dysregulation syndromes related to recovery of immunity on cART. HIV-mediated CD8 encephalitis is a rare neurological syndrome due to perivascular inflammation caused by infiltration of CD8+ cells.

Methods & Materials: We report the clinical and pathological features of three cases of CD8 encephalitis which will sensitise the clinicians to have high index of suspicion to recognize this entity early.

Results: All three patients were men with a mean age of 42.3 years. The mean duration of HIV was 10 years. The patient’s mean CD4 at presentation was 392 cells/microL and blood HIV viral load was 11,588.3 copies/ml. All patients presented with an average duration of 4 months with cognitive decline, especially memory disturbances and tremors. The mean cerebrospinal fluid (CSF) cell count was 65 cells/ml, lymphocyte predominant (mean - 97%), and protein was 171.3 mg/dl. The CSF lymphocyte subset analysis showed a median CD8 cell proportion of 54.7%. CSF was tested negative for viral infections (HSV1, HSV2, CMV, JC virus and VZV) and VDRL. The CSF cultures were sterile and GeneXpert was negative. MRI brain revealed diffuse hyper intensities involving the deep grey and white matter and typical perivascular hyperintensities were present in one patient. All patients were started on steroids after excluding other etiologies. Two patients made a complete recovery while one patient in whom the diagnosis was delayed succumbed to the illness. Postmortom brain biopsy of the patient who had a fatal outcome demonstrated perivascular cuffing with lymphocytes positive for CD8 and negative for CD4 and CD20 markers on immunohistochemistry, consistent with CD8 encephalitis.

Conclusion: CD8 encephalitis is a rare but potentially treatable cause of cognitive decline in patients with HIV on cART. The typical presentation includes memory impairment with extrapyramidal symptoms like tremors with lymphocytosis in CSF and predominant CD8 cells in CSF. MRI may show the typical perivascular hyperintensities in the deep brain tissue. Aggressive early steroid treatment after excluding opportunistic infections is likely to result in complete recovery.
The level of education affects CD4 cell count and wellness among HIV infected adult between age group 18 to 60 years.

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Background: HIV infection continues to be one of the dominating infectious diseases with 36.9 million cases worldwide. However, HIV prevalence could be much more as only 51% of people living with HIV know of their status. Profound immunodeficiency is the hallmark of HIV infection which primarily occurs due to decrease in CD4 cell count. Anti Retroviral Therapy (ART) is initiated when CD4 count falls below 350/mm³ and it goes lifelong. The CD4 count could be influenced by various factors, some of which could be modified by the people living with HIV. In this study, we evaluated the effect of education level among HIV infected adults on the CD4 count and HIV associated co-morbidities.

Methods & Materials: The retrospective study comprised of newly diagnosed HIV infected adults between 18 to 60 years visiting National HIV Reference Laboratory, All India Institute of Medical Sciences (AIIMS), New Delhi. The selected population was not on ART at baseline. The selected time period was the last four years (July 2011-June 2015). The statistical analysis was done at GraphPad Prism 5 software using two tailed chi-square test at 95% confidence interval.

Results: Among 2867 ART naive HIV infected adults between 18 to 60 years, 14% had no education, 47% had education up to school level, 19% had higher education, i.e., graduation or above and the education level of remaining percentage was not available. Significant difference was found in the mean CD4 count of those having no education than those having higher education, i.e., graduation or above. Decrease in CD4 count below 350/mm³ was more likely seen among those having no education in comparison to other groups (P=0.0001). HIV infected adults with no education were more prone to HIV infection associated co-morbidities particularly diarrhoea (P<0.0001; OR=2.112; RR=1.845) and tuberculosis (P=0.0025; OR=1.918; RR=1.503).

Conclusion: Our data shows that education seems to affect the CD4 count and health of HIV infected adults. Access to education provides better understanding of sanitary and hygiene practices. Higher education creates higher income and thus better access to nutritious food. Proper sanitation, hygiene practices and nutrition increases the CD4 count among HIV infected adults.
Feeling the pressure: Prevalence and risk factors associated with systemic hypertension among HIV infected children and adolescents

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\textbf{Background:} The widespread success of combination ART has contributed to increasing lifespans among the affected, and long-term complications associated with HIV infection and ART are becoming increasingly apparent. Hypertension is a well characterized risk factor for future cardiovascular disease. Only one study has previously described the prevalence and risk factors associated with hypertension in HIV infected children and adolescents. We aimed to study these in a cohort from south India.

\textbf{Methods & Materials:} Children and adolescents attending the pediatric HIV clinic at Nireekshana-ACET in Hyderabad were invited to participate in the study. Clinical and laboratory data were collected through medical chart review. Anthropometry was recorded using standard equipment. Variables suspected to contribute to hypertension including body mass index (BMI), active/passive tobacco exposure, medical comorbidities, ART or other medication use were recorded. Blood pressure was measured using a standard clinical sphygmomanometer with appropriate bladder cuff size. Hypertension was defined as average systolic BP and/or diastolic BP that was $\geq$95th percentile for gender, age, and height on $\geq3$ occasions, while pre-hypertension was defined as average SBP or DBP levels $\geq$90th percentile but $<$95th percentile.

\textbf{Results:} Among 97 children who participated in the study, mean age was 11.2±2.8 years (range 3-18); 51 (52.5%) were males. All were perinatally infected, 88 (90.7%) were in early stages of disease (WHO clinical stage 1 & 2); mean CD4 count was 771.31±383.61 cells/mm$^3$. Two-third were on ART (mean duration 3.68±3.22 years). Around half (53) the children had normal BMI, 44 (45.3%) were underweight and one child was overweight. None of the children had exposure to tobacco smoke, alcohol, steroids or any BP elevating medications. Two children had past history of urinary tract infection. Only 2 children (2%) were found to be pre-hypertensive, and none (0%) were hypertensive.

\textbf{Conclusion:} Our findings differ from those reported by a previous study from the USA, which reported high rates of hypertension (20%) and pre-hypertension (15%) among infected children and adolescents. Genetic, lifestyle and anthropometric differences could be reasons for this. Since data are scanty, larger multi-centric studies are needed to quantify and qualify the burden of hypertension and future cardiovascular disease among children living with HIV.
Disclosure of HIV status: Perspectives from infected children in India
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Background: Following the widespread uptake of highly effective ART, young children living with HIV are entering adolescence and beyond, necessitating disclosure of HIV status. Although national and international guidelines advocate full disclosure, disclosure rates in India continue to remain low. We aimed to understand children's perspectives and experiences following full disclosure in the Indian context.

Methods & Materials: Children attending HIV clinics at 3 tertiary-care centres in South India were screened for full disclosure. Those who had full disclosure were further administered a pre-tested questionnaire assessing their knowledge about HIV, ART, perceptions following disclosure, stigma and ideas about their future. Disclosure was considered as 'full' when it involved the child being told that he/she has HIV specifically.

Results: A total of 247 caregiver-children pairs participated in the study. Only 24 (9.7%) children were reported to have full disclosure. The mean age of these children was 14.02±1.8 years (range 10-18); 14 were males. A parent was the primary caregiver for 16 children. Mean age at disclosure was 11.2±2.18 years (range 7-15). Medical personnel had disclosed to 14 children, while parents were responsible for disclosure to only 3 children. Disclosure was met with a sense of relief and acceptance by one-third of the children, and 21 children subsequently disclosed their status to others. Despite full disclosure, 5 did not know how HIV spreads and 3 did not know how infection could be prevented. Notwithstanding their positive status, most children felt that they were treated well at school and by their immediate relatives, although 11 children mentioned that they needed to hide while taking ART. Over 75% were hopeful of meaningful lives ahead. None of the fully disclosed children reported suboptimal adherence to ART.

Conclusion: Disclosure rates in India continue to remain low. Most of the fully disclosed children were comfortable to learn about their status, received good support from their immediate family and community and exhibited a positive outlook towards their future. The knowledge about HIV despite full disclosure was found to be inadequate, and parents seem to depend on medical personnel for disclosure. Future guidelines need to address these gaps to build on potential gains from full disclosure.
Association of anti retroviral therapy with changes in peripheral arterial disease status and severity - a vision

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Background: HIV infection causes direct endothelial dysfunction or damage and premature atherosclerosis. HIV induced endothelial dysfunction may manifest as peripheral arterial disease (PAD). Understanding PAD scenario and its relation with antiretroviral therapy (ART) may help to improve quality of life and to prevent critical limb ischemia or amputation. Till date no follow up study has shown the effect of ART on PAD. This study explores association of ART with changes in PAD status and severity.

Methods & Materials: In this ART clinic based cohort study conducted at a tertiary care hospital in eastern India, 193 consecutive ART eligible HIV positive patients of age between 18-49 years underwent ankle brachial pressure index (ABPI) measurement before ART initiation and after one year of receiving ART to examine the effect of ART on PAD. ABPI values less than 0.9 or more than 1.4 at rest were taken as abnormal.

Results: Prevalence of PAD before the initiation of ART was 30.57%. The prevalence dropped to 12.95% after 6 months and to 3.62% after 1 year of treatment. Mean age of the patients whose ABPI improved was lower than those in whom low ABPI persisted (p value 0.05). ABPI improvement was significant among people who were asymptomatic, belonged to urban population, had normal BMI, good functional status, lower WHO stage and who had no history of tuberculosis (p value< 0.05).

Conclusion: Prevalence of PAD decreased significantly after one year of ART. Routine ABPI measurement and early initiation of ART may reduce PAD morbidity and improve quality of life.
Effectiveness of AIDS education program on nursing students’ knowledge and attitudes in Sri Lanka

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Background: Stigmatization and discrimination of HIV/AIDS patients is an important issue in health care institutions. Nurses, who play a key role in health care of people living with HIV (PLHIV), should be professionally competent in AIDS knowledge and AIDS attitudes. This is necessary to minimise the stigmatization and discrimination towards PLHIV and to protect patients’ dignity. In Sri Lanka, like in many other countries, nurses’ AIDS knowledge and attitudes are not satisfactory. Various interventions used to correct this in many countries had inconsistent results. Hence the need for good intervention prevails.

Methods & Materials: We assessed the effect of an educational intervention using Quasi-experimental control group pretest post-test model which was done on second year nursing students in National School of Nursing, Sri Lanka from January to March 2015. Recruits completed self-administered instruments of HIV/AIDS knowledge scale and a HIV/AIDS attitude scale at pre-intervention and post-intervention points and were randomized to experimental group (N=65) and control group (N=64). AIDS education intervention, which had six two hour sessions, was done for the experimental group over 5 weeks. Teaching strategies included lecturers, small group activities and discussions and testimony of PLHIV. After the intervention, post-test was held for both groups. Collected data were analyzed using SPSS WIN 18.0.

Results: Both AIDS knowledge (p<0.00) and AIDS attitudes (p<0.00) of the student nurses improved significantly after the intervention of AIDS educational program. In AIDS knowledge subdomain scale of HIV course and manifestations (t=-6.84, p<0.00), HIV Transmission (t=-7.28.44, p<0.00), occupational risk of HIV and prevention (t=-10.59, p<0.00) and management of HIV and anti-retroviral treatment (t=-12.81, p<0.00) all showed significant improvement in the experimental group. In the subdomains of the AIDS attitude scale, namely blame and judgment (t=-3.35, p<0.001), attitude towards imposed measures (t=-5.44, p<0.00) and attitudes in comfortable in dealing with a HIV/AIDS patients (t=-4.25, p<0.00) there was significant improvement in experimental group compared to control group who had only the traditional education program.

Conclusion: Intervention showed statistically significant improvement in AIDS knowledge and attitudes. This model could be the basis for a standard module on HIV/AIDS in student nurses’ curriculum in Sri Lanka and in other countries.
Background: Human Immunodeficiency Virus largely has contributed to infant and child mortality in Uganda today and Africa at large. Women have the potential to transmit HIV to their children during pregnancy, labor and post-natally; about 30-40% of these children acquire the infection in the postnatal period (http://womenchildrenhiv.org). To minimize postnatal mother to child transmission (MTCT) of HIV, Various feeding options have been tried but their success has been challenged because little is known about the mothers’ beliefs and knowledge about such measures, which thus calls for further investigations into this practice.

Methods & Materials: We did a descriptive cross sectional survey in a post conflict district (more than 2 decades of unrest) to assess the mothers’ knowledge and practice of modified infant feeding (MIF) in prevention of postnatal MTCT of HIV. We interviewed HIV positive mothers bringing their children to Young Child Clinic (YCC) and Early Infant Diagnosis clinic (EID) at Gulu Regional Referral Hospital (GRRH) from December 2012 to February 2013.

Results: Out of the 400 HIV positive mothers that were interviewed, 260 (65.1%) were aware of MIF but only 129 (49.6%) put the knowledge into practice

About 73.6%of the mothers were aware of initiating supplementary feeds at 6 months, 91.71% knew they were meant to wean off their babies at the age of one year; 67.4% were informed about replacement feeding. About Practice, 69.3% were breast feeding their infants at the right frequency, 59% introduced supplementary feeds at the right time and 93.5% weaned at the right time.

Conclusion: A good proportion of the HIV positive mothers (65.1%) were knowledgeable about MIF but very few (49.6%) were applying the knowledge which therefore means that there is still a high risk of MTCT of HIV. Therefore this means that apart from knowledge, other factors such as mothers’ attitude towards MIF, mothers’ income and other maternal illnesses other than HIV need to be studied.
CD4 pattern in HIV positive patients on HAART exposed to moringa oleifera leaf powder in south east Nigeria

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Background: HIV infects vital cells in the human immune system such as helper T cells (specifically CD4 T cells), macrophages, and dendritic cells (Cunningham et al, 2010). HIV infection leads to low levels of CD4 T cells through a number of mechanisms, including apoptosis of uninfected bystander cells (Garg et al, 2012), direct viral killing of infected cells, and killing of infected CD4+ T cells by CD8 cytotoxic lymphocytes that recognize infected cells (Kumar, Vinay, 2012). Moringa trees are edible and have long been consumed by humans and have also been advocated as an outstanding indigenous source of highly digestible protein. Moringa has been used to combat malnutrition. Moringa’s antioxidant and nutritional benefits cannot directly compete with the superior results of modern antiretroviral, even though it shows promise in providing reduced mortality rates and improved health for HIV-positive and AIDS patients.

Methods & Materials: A total of 40 (15 males and 25 females) consenting adult HIV Patients who had been on highly active anti retroviral therapy (HAART) for more than a year were enrolled into the study. The age range was from 18 to 65 years with a mean of 42.5 years. The design was longitudinal randomised convenience sample technique with pre and post treatment check up. Ethical approval was obtained from Nnamdi Azikiwe University Teaching Hospital. Subjects were exposed to 20g daily Moringa oleifera leaf powder (MOLP) mixed with any local meal prepared with palm oil daily for two months. The pre and post CD4 counts were analysed.

Results: The result showed a marked increase in the male post-test CD4 value of 496.1± 61.52 cells/mm³ when compared with the pre-test CD4 value of 362.7± 49.68 cells/mm³ [P<0.05 (0.0003)]. Also there was a significant increase in the female post-test CD4 value of 547.6 ± 57.9 cells/mm³ compared with pre-test CD4 value of 459.7± 40.65 cells/mm³ [P<0.05 (0.0031)]. Comparatively, there was a gender difference with the males (496.1 ±40.65) gaining more than the females (547.6 ±57.9) irrespective of the number that was enrolled (P<0.05).

Conclusion: Moringa oleifera has the potential of improving the CD4 count of HIV positive patients on HAART translating to better treatment outcome.
Malaria preventive practices and clinical burden among HIV patients attending clinic at a tertiary hospital in Nigeria


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Background: It is now three decades into the HIV epidemic which was superimposed on an uncontrolled Malaria burden. These two diseases are of major public health importance and clinical importance in Sub-saharan Africa and in Nigeria in particular. HIV and malaria co-infection is common yet surprisingly few clinical associations have been reported in Nigeria. This study aims to assess Malaria preventive practices and the clinical burden among HIV infected patients.

Methods & Materials: A cross-sectional descriptive study was conducted during the months of January and February 2014 among 356 HIV positive patients attending Nasara clinic of Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. Ethical approval was obtained from the Ethical review committee while written informed consent was obtained from all respondents. A semi-structured, self-administered questionnaire was used to elicit information from the respondents. Data was analysed using SPSS Version 21.

Results: About 99.2% of the respondents were familiar with the term Malaria while 90.2% of the respondents knew that co-infection exists between Malaria and HIV. However only 36.2% of the respondents had good knowledge of Malaria and its co-infection with HIV. Majority 93.3% of them had appropriate practice of Malaria prevention with 96.3% using one or more methods of prevention and 78.7% use a prophylactic antimalarial drugs. More than half (54.2%) of the respondents had at least an episode of malaria in the last six months while 72.8% have had limitations from carrying out their normal activities.

Conclusion: This study emphasized that despite a good knowledge of malaria and its preventive practices among respondents, malaria still remains a common problem faced by HIV patients resulting to a substantial limitation from carrying out normal physical activities. It is suggested that the government should initiate, encourage and sustain focused based campaigns about Malaria and HIV control mechanisms as part of concerted efforts to curtail both infections.
Elite neutralizers among HIV-1 Subtype-C infected individuals from southern India

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**Background:** The isolation of broadly neutralizing antibodies has been a high priority since they were identified as potential targets for passive immunotherapy to slow or halt the disease progression in HIV-1 infected individuals. Until recently, only a few recombinant NAbs were available for clinical trials and requirement for the identification of broadly neutralizing sera from HIV-1 infected individual is still high. Although a few human broadly neutralizing antibodies to HIV-1 exist, these antibodies have limited reactivity against non-clade B viruses and so far none of the broadly neutralizing antibodies isolated from Indian donors. Here we attempt to identify cross reactive neutralization response among patients infected with HIV-1 subtype C from southern India.

**Methods & Materials:** We studied HIV-1 neutralizing antibody (nAb) response in serum among 23 HIV-1 chronically infected individuals with median CD4 Count 581(IQR 339-786) and Plasma viral load 15506 (IQR 6944-51308). These individuals were diagnosed for HIV between 6 and 10 years and naïve for antiretrovirals. Neutralization was measured in a single round infection assay in TZM-bl cells using 19 Tier-II Multi-clade envelope pseudotyped viruses (Subtype A,B,C,AE,BE & G) representing from global virus panel. Correlation and Neutralization breadth was analyzed using Spearman’s rank test and p value less than 0.05 was considered statistically significant.

**Results:** Broad and potent neutralization (ability to neutralize 75% of pseudoviruses) was observed in 5 (22%) individuals (Figure:1). Interestingly 3 individuals have shown geometric mean neutralizing titer over 500 and are termed as “elite neutralizers”. The overall neutralization breadth was positively correlated with HIV-1 RNA (rho = 0.362, p=0.0089) and negatively correlated with CD4+ T Cell counts (rho = -0.667, P=0.0004).

**Conclusion:** This study has witnessed elite neutralizers with heterologous 'Tier II viruses' from southern India. It is evident that broadly neutralizing specimens can be identified with larger studies with further extension to define epitope specificities, there by identifying potential antibodies and epitopes for use in therapeutic and vaccine trials.
DC-SIGN and L-SIGN repeat-region polymorphisms influence HIV-1 disease progression in slow and rapid progressors among perinatally-infected children in India

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Background: Among human host factors known to modulate HIV disease progression, the C-type lectins, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin, DC-SIGN and the liver/lymph node-specific L-SIGN have been poorly described in children with HIV. We aimed to study the exon 4 repeat-region polymorphisms, and assess the impact of DC/L-SIGN homozygous and heterozygous genotypes among pediatric slow and rapid progressors.

Methods & Materials: We defined slow progressor children as perinatally-infected, asymptomatic, with known HIV infection for >10yrs, ART-naïve, and CD4>500 cells/mm³ and rapid/normal progressors as HIV infection<10 years, on ART, CD4<500 cells/mm³. Genomic DNA isolated from whole blood was genotyped for DC/L-SIGN exon 4 tandem repeat-region variants by PCR.

Results: Among 25 slow progressors included, mean age was 10.8±2.42 years, 64% females, median CD4 count was 751 cells/mm³ (IQR 575-1036.75) and viral load, 4.36 log copies/ml (IQR 4.09-4.84). Among 40 rapid progressors, mean age was 6.07±2.4 years, 45% females, median CD4 was 462.5 cells/mm³ (IQR 291-663) and viral load, 5.42 log copies/ml (IQR 4.97-5.95). The exon-4 repeat-region DC-SIGN polymorphism was not observed in our population as all samples showed 7/7 homozygous DC-SIGN genotype. In the L-SIGN repeat region, eight genotypes were found. The total L-SIGN heterozygosity in slow (7/5, 7/6, 9/5, 9/6 and 9/7 genotypes) and rapid (6/5, 7/5, 7/6, 9/5, 9/6 and 9/7 genotypes) progressors was 76% and 45% respectively (P=0.02; OR=3.87; 95%CI 1.3-11.7). Rapid progressors had a significantly higher occurrence of 7/7 homozygous L-SIGN genotype (P=0.03; OR=3.5; 95%CI=1.16-10.6). The L-SIGN allele frequency in slow progressors was 14% for allele 5, 14% for allele 6, 54% for allele 7 and 18% for allele 9. L-SIGN alleles 6 and 7 were significantly more frequent in rapid progressors than slow progressors (P=0.03, OR=2.24, 95%CI=1.07-4.72 respectively).

Conclusion: This is the first study to determine the DC-SIGN and L-SIGN polymorphisms in HIV-1 positive children in India. While heterozygous DC-SIGN genotypes were not seen in this population, the higher prevalence of heterozygous L-SIGN genotypes among slow progressors suggests their protective role in HIV disease progression among children in India.
CD4 Levels >350 cell/µl at initiation of option B+ Predict retention in care amongst mothers in urban health facilities in Uganda

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**Background:** Uganda like most countries is providing Option B+ to HIV+ mothers. However, patients who start ART with a high immunity are often thought to be poorly retained in care (Tayler et al, 2010). Poor retention in care predicts poorer survival with HIV infection (Thomas et al, 2007). Patients with CD4 >350 cells/µL are asymptomatic and presumed to be comfortable with their status quo. The study hypothesized that CD4 <350 cell/µl at initiation of Option B+ would also predict higher retention compared to CD4 >350 cell/µl.

**Methods & Materials:** A retrospective cohort study was done on mothers that were initiated on Option B+ between December 2012 and January 2013 in six urban public health facilities in Uganda. Patients were observed starting at Option B+ initiation to patient outcome such as at death, transfer out, loss to follow-up and 12 months after initiation. Only mothers whose CD4 was recorded at initiation of Option B+ were considered for analysis.

**Results:** A total of 601 mothers with mean age 25.0 years (95% CI: 21-28) were enrolled and of these 65% were still in care one year after option B+ initiation. At the time of enrollment, 313 mothers had CD4 > 350, 74 had CD4 <350 while 212 had no CD4 done. Majority, (80%) mothers had no prior exposure to ARVs at the time of initiation. There was a significant relationship between retention and prior treatment, age of mother > 20yrs, and CD4 levels. After regression modelling, the odds of being in care among CD4 >350 were 2.21 (p=005) times more likely to stay in care compared to those with lower CD4. There was no significant relationship between retention and age or prior ART.

**Conclusion:** Contrary to the study hypothesis and knowledge among the general HIV patients, our study revealed that women started on option B+ with CD4 >350 cell/ul were 2.21 times more likely to stay in care, one year after ART initiation.
An analysis of global HIV prevalence among refugees, asylum seekers, and migrants, using the US Bureau of the Census databank

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**Background:** As a risk-factor population for HIV infection, unsettled populations, in particular, refugees and migrants are understudied.

**Methods & Materials:** Using the US Bureau of the Census database, all studies listed of high quality with keywords “refugee,” “asylum,” “immigrant,” or “migrant” were collated. From this cohort, inapplicable and studies were deleted (eg, those listed as “non-migrants”). The data were analyzed using STATA 11, with respect to HIV prevalence, median year of collection, sample size, and age.

**Results:** There were 645 eligible studies from 40 countries carried out between 1987 and 2015 deemed adequate for analysis. Most studies were from Asia (70%) or Africa (24%). The overall HIV prevalence among unsettled populations was 6.5% (SD 9.8, median, 2.5%). Over the last 5 years, among 446 studies, the mean prevalence was 6.5%. The 151 studies among only females showed a higher prevalence than those among only males (8.4% vs 6.4%, P =0.43).

The sample size of 95 studies with data ranged from 5 to 930 persons, mean 359 (the mean HIV prevalence for this subset, 11.5%). Studies with a smaller sample size tended to show higher HIV prevalence (correlation coefficient, -0.52). There was a small increase in prevalence through time (correlation coefficient, 0.05). Studies before 2001 had a median prevalence of 7.7% while those after 2001 showed a median prevalence of 6.3% (P = 0.0001, Kruskal Wallis. Age values were available for only 72 studies, with a mean prevalence of 10.5%, and slightly increased with age, correlation coefficient of 0.28. A regression analysis of age, sample size, and time of study against prevalence had only 24 studies but showed that only time of study as significant (P = 0.009, adjusted R square, 0.57).

**Conclusion:** These data on globally unsettled populations show that while studies with small sample size, of females, and of older age populations show a higher HIV prevalence, the key factor remains time of study, with a slow increase in HIV prevalence through the interval analyzed, 1987-2015. With a mean recent prevalence of 6.5%, it is important that HIV prevention activities be directed toward unsettled populations, regardless of other risk factors.
Mitochondrial dysfunction among HIV-1 infected patients of South India and evaluation of mitochondrial DNA as a biomarker of mitochondrial toxicity

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Background: NRTI class of drugs though they cause mitochondrial toxicity, owing to their cost is the preferred class of drugs in resource-limited settings like India, hence there is a greater need to identify a novel biomarker of mitochondrial toxicity. In this study mitochondrial dysfunction was estimated from peripheral blood mononuclear cells (PBMCs) and the utility of mitochondrial DNA (mtDNA) as a marker of mitochondrial toxicity was evaluated.

Methods & Materials: In this longitudinal observational study, 40 HIV -1 infected patients treated with NRTI based regimen and 57 HIV -1 infected ART untreated patients were followed for 18 months at an interval of 6 months and data were compared with 24 HIV uninfected controls. Mitochondrial dysfunction was determined from mtDNA content by real time PCR, mitochondrial membrane potential damage (total lymphocyte ∆Ψmlow) by flow cytometry and ND1gene of mtDNA was sequenced.

Results: Among the HIV infected, mitochondrial dysfunction was more pronounced in the ART untreated than the treated. mtDNA content and total lymphocyte ∆Ψmlow (p=<0.0001) were significantly different among the HIV infected than the controls. Out of the 22 mtDNA variants observed in HIV infected individuals, 27% were found to be associated with mitochondrial mediated pathogenic conditions, distributed in 10% of on ART and in 8% of ART untreated. mtDNA content was significantly(p<0.0001) reduced in both the HIV infected groups, ART untreated had 83.3(65.56-113) and treated had 92.53(68.64-126.7), than the controls(127.5(110.6-167.7)) throughout the follow up, however it did not differ significantly among the HIV infected. Analysis of mtDNA content in relation to the adverse events, though differed significantly at the first (p= 0.014) and second visit (p= 0.029) was increased in the subsequent visits among the patients symptomatic for toxicity.

Conclusion: Mitochondrial dysfunction is evident among both the treated and untreated HIV-1 infected patients of South India. As mtDNA depletion failed to relate consistently with laboratory adverse events during the complete follow up, measuring mtDNA content in PBMC alone may not be sufficient enough to predict or monitor changes in NRTI associated mitochondrial toxicity.
Pre-exposure Prophylaxis (PrEP): Attitudes, preferences and risk compensation behavior among men who have sex with other men (MSM) in India

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Background: The HIV epidemic in India is concentrated among High Risk Groups and continues to be high among MSM population. Pre-exposure prophylaxis (PrEP) has shown promising results in recent trials. The potential of PrEP as an effective HIV preventive strategy among MSM remains to be studied in India.

Methods & Materials: A structured questionnaire was administered to MSM selected by snowball sampling technique. Individual risk behaviours and demographics were categorised as high, moderate and low risk for HIV and a composite risk score was calculated. To assess the agreement between calculated and self-perceived risk, kappa statistic was used. Bivariate logistic regression was used to ascertain determinants of willingness to use PrEP and to study the anticipated drift in risk compensation behavior with PrEP use.

Results: Data from the initial 55 respondents of this multi-city study are analyzed. The average respondents were predominantly Kothi (31%), found their partners online (83%), had three or more sexual partners in the previous three months (44%), had anal sex less than once a month (45%), used condoms all the time while having anal sex (55%), and considered HIV a risk for them (67%). There was no agreement between self-perceived risk for HIV and calculated composite risk (Kappa= -0.2182, p-value>0.05). Majority (87%) reported willingness to use PrEP which was strongly associated with self-perceived risk of HIV (OR=5.70, p = 0.009). Willingness to use PrEP remained high even after learning about the need for concurrent condom use (OR= 10.83, p = 0.009), regular HIV tests (OR= 30, p = 0.003) and a daily dosing schedule (OR = 12.19, p =0.007). Individuals with low calculated composite risk did not show an increase in anticipated risk compensation behavior with PrEP use (OR=0.23, p = 0.047).

Conclusion: MSM in India show high willingness to use PrEP, even if they have to use condoms in combination with PrEP, or be regularly tested for HIV, or have to adhere to a daily dosing schedule. Contrary to major concerns, risk compensation behavior would not increase with the advent of PrEP. Our findings show that PrEP uptake could be considerable whilst not increasing compensatory risk behavior among MSM.
Background: The advent of Highly Active Antiretroviral Therapy (HAART) has reduced HIV/AIDS related morbidity and mortality, so more people with HIV/AIDS live longer. This study aims to determine the health related quality of life of HIV/AIDS patients on HAART at Ahmadu Bello University Teaching Hospital (ABUTH) Zaria.

Methods & Materials: A cross-sectional descriptive study among 384 HIV patients on HAART at ABUTH was conducted in April 2015 with the sample obtained by a systematic sampling process. A structured, interviewer administered questionnaire was used to assess the health-related quality of life of the respondents and the data was analyzed using SPSS Version 21. Ethical approval was obtained from the Ethical review committee while written informed consent was obtained from all respondents.

Results: Majority of the respondents were females (64.3%), with about 50% in stage 1 of the disease, 40% in stage 2 and the remainder in stage 3. About 55% had CD4 count of 200-500 cells/mm$^3$, 34% had greater than 500 cells/mm$^3$ and 11% had less than 200 cells/mm$^3$. Only 7.8% of respondents were dependent on Alcohol and smoking while 79.6% were still engaged in paid work. A total of 22.9% of respondents had lack of energy while 17.2% experienced pain and 15.6% had drug allergy.

The domain with the lowest score was the psychological domain (5.99), followed by the social domain (6.27), the physical domain (7.89) and the socio-demographic domain (38.46) which is the domain with the highest score. There was statistically significant relationship between CD4 count of respondents and their clinical stage (P < 0.0001), but no statistically significant relationship between age and suicidal thoughts, age and suicidal attempt, sex and suicidal thoughts and between sex and suicidal attempt (p= 0.05).

Conclusion: Majority of respondents had low quality of life in the psychological, physical and social domains and high quality of life in the socio-demographic domain of the health related quality of life (HRQOL). These findings highlight the need for enhanced psycho-social support, effective management of symptoms and a better environment for improving health related quality of life among people living with HIV/AIDS.
Current problems in serologically based diagnostic algorithm of HIV 1/2: The re-evaluation of immunodot blot assays in HIV 1/2 verification in Turkey


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Background: The use of conventional (serologically based) HIV 1/2 diagnostic algorithm is controversial in recent years. In this study, we aimed to evaluate the serum samples of patients that have been sent to verification tests because of repeat reactive ELISA results and showing HIV1+HIV2 positive band pattern and also to evaluate the position of Western Blot/Line-Immunoassay (WB/LIA) verification tests on the national HIV 1/2 diagnostic algorithm.

Methods & Materials: This study was planned as a cross-sectional/retrospective study (January 2014 – September 2015) in serum samples of patients who were referred to the Dermatological Venereal Diseases Hospital, Cerrahpasa Faculty of Medicine and Turkish Red Crescent North Marmara District Blood Centre. The reactivity of repeat ELISA results has been verified with WB/LIA assays in accordance with the national algorithm. The verified serum samples were confirmed with nucleic acid (NAT) based assays.

Results: In the study, 3224 of 10.591 samples with repeat ELISA reactivity (30.44%) were verified by WB/LIA for HIV infection. In 32(0.99%) of the verified serum samples, along with HIV1 bands HIV2 gp36 bands were also detected positive. Only, 17 of the verified 32 serum samples with gp36 bands were repeated and no gp36 band positivity was detected by Bio-Rad Genius HIV 1/2 Confirmatory Assay in this 17 serum samples. Moreover, the HIV2 RNAs of these samples were also detected as negative. Therefore, the HIV1+2 co-infection possibility in these patients has been excluded. All of the serum samples of 32 cases were HIV1 RNA positive. The remaining 15 cases were not attainable because of various reasons.

Conclusion: The detection of false gp36 band in HIV1 infections cause problems in the diagnoses of HIV1/2 patients. These problems in WB/LIA tests may cause delays in the diagnoses of patients and therefore negatively impacts their psychological state. In this respect, we suggest that the WB/LIA results have to be evaluated from this aspect and the addition of assays that can produce faster results (peptide-based immunochromatographic methods that distinguish HIV1/2, NAT) to Turkey’s diagnostic algorithm as present in CDC algorithm may be suitable in these situations.
Uptake of intermittent preventive therapy among pregnant women attending antenatal clinics in public and registered private health facilities in Oyo State, Nigeria

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**Background:** Malaria infection during pregnancy remain a major public health problem in Nigeria. The 2013 Nigeria Demography and Health Survey revealed that while most pregnant women (PW) access Antenatal Care (ANC) from skilled care providers, majority of them do not benefit from interventions to prevent malaria. Intermittent Preventive Therapy (IPTp) using Sulphadoxine-Pyrimethamine (SP) is a full therapeutic course of intermittent medicine given to PW at routine ANC visits. The WHO recommends that this treatment be given to all PW at each scheduled antenatal care visit except during the first trimester. In Nigeria, the national guidelines and strategies for malaria prevention and control during pregnancy has been revised to reflect WHO recommendations.

**Methods & Materials:** This study utilized secondary data from the routine national District Health Information System which houses the health management information system to assess IPTp uptake among PW who attended ANC in public and private health facilities (HF) in Oyo State Nigeria from October 2014 to September 2015. The national data system is able to report only two doses. Descriptive statistics was performed to assess IPTp uptake from reporting HF within the period.

**Results:** A total of 122,320 pregnant women attended antenatal clinics in 1139 public and private HF; reporting rate was 93.3% for all health facilities; 99% for public and 84% for private during the period. Overall, 54% (75.1% public, 28.4% private) of first ANC attendees received IPTp1; while only 20% (75.5% public, 24.9% private) received IPTp2. Sixty-four percent attended ANC for a minimum of four times; 53% of all PW who attended ANC were delivered in the HF (48.8% public and 51.2% private).

**Conclusion:** Although majority of the PW had attended ANC at least four times, uptake of the two doses of IPTp remains low; this was much worse in the private HF. Targeted capacity building for ANC providers and HF in the private sector may reduce missed opportunities for prevention of malaria among women attending ANC in the state. Further exploration of kind of care received by those who attended ANC but did not deliver in the HF is recommended.
Prospective cohort study on rectal colonization with Carbapenem Resistant Enterobactericeae in patients admitted in a tertiary care hospital
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**Background:** The data on prevalence of Carbapenem Resistant Enterobactericeae (CRE) colonisation in south India is lacking. This study was carried out to assess the prevalence of rectal colonisation of CRE, to compare CRE prevalence in patients admitted from the community with those with prior healthcare exposure and association between CRE colonization and true CRE infection.

**Methods & Materials:** 138 consecutive patients admitted to multi disciplinary ICU from May 2015 were included after appropriate exclusions. Rectal swab was collected within 24 hours of admission by trained nurses. Data was collected in a structured proforma, samples were processed according to CDC guidelines. Patients were followed up till discharge. Based on the published data, sample strength of 114 was calculated for adequately powering the study at 95% CI and 20% desired precision. Fischer’s Exact Test was used for analysing the statistical significance.

**Results:** 138 patients were included in the study and 9 (6.5%) were found to have rectal colonisation with CRE. K. pneumoniae was isolated in 7 cases and E.coli in 2. Mean age was 51.78 years. 63% were males. 63% had comorbidities, diabetes mellitus being most common (n=72). 61% had previous hospitalisation within last 3 months. Previous antibiotic use, especially carbapenem use, was significantly associated with CRE colonisation (p=0.004, OR-7.59,LL-0.93,UL-61.63 after correction and p=0.026,OR-8.71,LL-1.79,UL-42.3 respectively). Out of 138, 20 patients grew CRE from sites other than rectum, of which 6 had true infection, while 14 were colonised. Rectal colonisation with CRE was found significantly associated with isolating CRE from other samples (p=0.019, OR-5.65,LL-1.37,UL 23.27) though not with true infection [p=0.662,OR-2.27,LL-0.24,UL-21.02(after correction)]. A logistic regression was performed to ascertain the effects of variables on the likelihood that participants have CRE colonisation and was statistically significant, $\chi^2=27.27$, $p =0.039$. The model explained 46.9% (Nagelkerke $R^2$) of the variance in CRE colonisation and correctly classified 94.9% of cases.

**Conclusion:** The prevalence of CRE rectal colonisation is low compared to previous studies. Understanding the risk factors like previous hospitalisation, carbapenem use and the risk of multiple site colonisation will help focusing infection control measures in these patients to prevent transmission.
Evaluation of antimicrobial and disinfectant resistant bacteria isolated from the environment of a University Health Centre  
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**Background:** The hospital environment continues to be a source of healthcare associated infections despite hospital infection control strategies. In addition there is an increased prevalence of antimicrobial and disinfectant resistant bacteria. This study was carried out to detect the presence of antimicrobial and disinfectant resistant pathogenic bacteria after the disinfection of the floors and surfaces in a University health centre in Nigeria.

**Methods & Materials:** Environmental samples were obtained from different areas within the health centre such as the wards, theatre, treatment room, laboratory and the waiting area. Samples were taken from floors, door handles, patient beds, floors, table tops using moist sterile swabs. Samples were inoculated on appropriate culture media and isolates were identified using standard bacteriological methods. Antimicrobial susceptibility and disinfectant susceptibility tests were carried out using the disk diffusion and agar dilution techniques.

**Results:** The isolated organisms include *Staphylococcus aureus*, coagulase negative staphylococci, *Klebsiella* spp, *Bacillus* spp, *Escherichia coli*, *Proteus* spp and *Streptococcus* spp. Antimicrobial resistance was observed against penicillin, trimethoprim-sulphamethoxazole, tetracycline, gentamicin and cefoxitin. Multidrug resistance was observed in *S. aureus*. Susceptibility to disinfectants varied with concentrations but there were resistance to certain disinfectants at the recommended dilutions.

**Conclusion:** This study has shown that antibiotic resistant and disinfectant resistant organisms may persist in the environment after disinfection. Continual monitoring of the hospital environment is necessary to prevent infections caused by resistant pathogens.
Tetanus and use of magnesium in resource limited country

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**Background:** Tetanus is a nervous system disorder characterized by muscle spasms that is caused by the toxin-producing anaerobe, Clostridium Tetani. It is almost obsolete disease in developed country but one of the most neglected diseases in developing country.

**Methods & Materials:** Case series. Here we describe the 320 cases of diagnosed tetanus cases admitted in Infectious unit of Mymensingh Medical college Hospital during the period of 3 year from July2011 to July 2014.

**Results:** Median age of the patient was 40 (R-3-70) years and 264 (82.5%) were male; outnumbered were female. Most 95%(304) patients were from rural area and 65%(208) had family income around 100 US$/month. Clinical pictures revealed almost all (95.7%) had lock jaw, pain in the neck and back. About 47.8% patinet had resus sardonnicus and 28% had opisthotonus. 87% had both upper and lower limb spasticity. One hundred twenty four (38.75%) had history of wound in lower limb. 21.6% (69)had upper limb and 20 had history of CSOM, 10 had incomplete abortion, 16 had due to surgical complication. Large number of patients(81) failed to reveal any causative factor. Incubation period between wound and tetanus developed from 6-90 days. Inj. TT and TIG(1500 to 3000IU) was given intramuscularly as a single dose in all patients. All patients were treated with Inj. Metronidazole and occasionally with other add in antibiotics. Spasm was mostly controlled by continuous infusion of Inj. Diazepam ranging from 30mg to 100mg/day. Inj Magnesium sulphate heptahydrate BP 4% w/v in 100ml i.v. solution was used 8 hourly as an additional therapy for control convulsion. Parenteral feeding followed by late oral feeding was given to maintain nutrition. Sixtysix (20%) patient died mostly due to lack of ICU support , 15 recovered with sequele. Most of the patients (75%) died within 3 days of treatment(R-1-15days). Most patients(87%) had lack a history of receipt of full series of tetanus toxoid immunization and inadequate prophylaxis following a wound.

**Conclusion:** Low dose of magnesium is a good choice for treatment of tetanus; where modern logistic support is almost nills. Prevention of tetanus is a real priority subject in our community health.
The role of toxin-antitoxin systems in the survival of multidrug tolerant pathogens and designing of new approaches to treat them

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**Background:** The chronic infections are often caused by pathogens that are susceptible to antibiotics. Existing antimicrobial therapies are not able to treat or eradicate chronic infections. Recent studies show that treatments for chronic infections are failed due to small subpopulation of microbial cells, called persister cells. Persister cells have ability to survive even at lethal dose of antibiotics known as multidrug tolerance. The molecular mechanism of persister cell formation is unknown and not well understood but it is well established that toxin-antitoxin systems play a key role in the formation of same. Currently available therapeutic suppress, but do not eradicate the infection completely. An effective method for their eradication is great interests to researchers. We found three gene loci in the genome of *Pseudomonas aeruginosa* codes for toxin-antitoxin proteins. Certain metabolites if given in combination of antibiotics, they can treat persister cells more efficiently. The identification and evaluation of toxin-antitoxin genes can provide a clue to develop new strategies for treating pathogens. The study of various metabolites effects in combination with antibiotics on persister cell can lead to development of new antipersister therapy for treating chronic and latent infections.

**Methods & Materials:** Persister Cell Assay (Antibiotic based): Culture of *P. aeruginosa*, ampicillin & LB-Agar. Persister Cell Assay (Green fluorescent protein based): Arabinose inducible green fluorescent protein containing vector. Protein-Protein Blast: Putative toxin-antitoxins operon from the genome of *P. aeruginosa* using NCBI server. (http://blast.ncbi.nlm.nih.gov/Blast.cgi) Phylogenetic Analysis: Different annotated protein sequences were multiple aligned and phylogenetic trees were constructed by using CLC Genomics Workbench (version 5.5 software).

**Results:** Please find attachments.
**Conclusion:** This experiment revealed a small subpopulation of cells that remain alive irrespective of concentration of antimicrobial agents. In exponential phase the number of persister cell was low than stationary growth phase populations. In addition, the persister cells of *P. aeruginosa* exhibit a high tolerance to the variety of antibiotics, and phenotype was not inherited as tested with four passages of *P. aeruginosa* populations. *In-silico* results show the presence of *parDE*, *relBE*, and *higBA* toxin-antitoxin systems among which *ParE* toxin generates double strand breaks while *RelE* & *HigB* toxins induce mRNA cleavage.
Impact of antimicrobial stewardship in collaboration with infection control on hospital-acquired infection rates in a subspecialty cancer treatment facility

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Background: Antimicrobial stewardship programs (ASP) in collaboration with infection control have been shown to reduce rates of hospital-acquired infections (HAI) and prevent development of resistant organisms. ASP ensures the medical staff is appropriately educated on protocols developed by infection control and monitors the use of antibiotics to reduce the risk of *C. difficile* and other multi-drug-resistant organisms. These services are imperative in hematology/oncology patients who are at elevated risk for acquiring HAIs due to immunosuppression, chronic intravenous access, and frequent hospitalizations. We sought to determine if ASP in combination with infection control could reduce HAIs in a subspecialty oncology hospital.

Methods & Materials: We performed a retrospective review of HAIs between January 2012 and September 2015. Antimicrobial usage from July 2011 through September 2015 and antimicrobial susceptibility data from December 2010 through November 2014 was collected. During this time, ASP and infection control strategies included: nursing education on appropriate methods of accessing ports and management of foley catheters, adherence to strict hand-hygiene protocols, consultation by the infectious disease physician, and daily review of all patients on antimicrobial agents by the infectious disease pharmacist.

Results: In a 74-bed subspecialty cancer treatment facility with oncology and stem cell transplant patients, ASP was established in 2012. Since inception, the rate of central line-associated blood stream infections (CLABSI) steadily declined and was maintained below the national pooled mean since May 2013 (Figure 1). Additionally, no incidences of CLABSIs were reported for 1 year (January 2014 through February 2015). Due to the low usage of meropenem and imipenem (24 and 29 days of therapy per 1,000 patient days respectively) between August 2011 and September 2015, there have been only 3.2 isolates per 1,000 patient days identified of *Carbapenem-resistant Enterobacteriaceae*. Additionally, rates of HAI MRSA and *C. difficile* remained extremely low (0.18 and 0.2 infections per 1,000 patient days, respectively) after ASP was implemented.

Conclusion: Through education, implementation of specific protocols, and ASP interventions, the risk of hospitalized hematology/oncology patients acquiring HAI infections and antimicrobial resistance has been significantly reduced. This intervention affords this high risk population the opportunity to pursue cancer-specific treatment with a reduced risk of developing infectious complications.
Inhibition of Japanese encephalitis virus infection by biogenic catechin silver nanoparticles: An in-vitro study

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Background: The interaction of nanoparticles with microorganisms is an emerging field. Within this, an area that has been limitedly explored is the interaction of nanoparticles with viruses. Recently studies on silver nanoparticles (AgNPs) showed potent antiviral activity against HIV, HBV, influenza and dengue virus. However, synthesis of metallic nanoparticles involves physical and chemical approach utilizing toxic chemicals, which may hinder its application for human use. Therefore, development of novel eco-friendly nanoparticles is of great interest. In the present study, we synthesized green AgNPs with catechin as reducing agent. Catechin, a polyphenol being itself having antiviral property may enhance the efficiency of AgNPs. We evaluated biogenic catechin AgNPs against Japanese encephalitis virus (JEV), which is a major public health problem particularly in Asia.

Methods & Materials: Green nanoparticles were synthesized by adding purified catechin into AgNO3 solution by following standard method. Standard circulating JEV strain JERG07 (Source: Human, 2007, Assam, India) was used. In vitro assays were done using VERO cell line. Cell viability assay for 2 fold diluted green AgNPs was done using MTT standard protocol assay. Cytopathetic effect (CPE) inhibition assay was performed with 1 Multiplicity of infection (MOI) of JEV. Cells were also observed daily under inverted microscope and were assigned a score based on appearance of CPE. Further, virus yield reduction assay was done to quantify the level of protection of nanoparticles treated both at pre & post JEV infection.

Results: Transmission Electron Microscopy (TEM) analysis revealed formation of biogenic AgNPs within the size of 50nm. Maximum (100%) cell viability for green nanoparticles was observed in the range from 0.04µg/ml to 5.85 µg/ml. Within the same range CPE inhibition assay also showed full protection against JEV. Further, virus yield reduction assay showed reduction in plaques number in comparison to virus control in both pre & post JEV infection cells.

Conclusion: The present study demonstrated the ability of catechin reduced biogenic AgNPs to prevent JEV infection by inhibition of virus attachment and post infection spread. Future studies should be focused on elucidating the mechanism of nanoparticle-virus interaction at cellular and microscopic level.
Point surveillance of staphylococcus aureus nasal carriage among health care workers in rural tertiary care center of Central India
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Background: The anterior nares have been shown to be the main reservoir of Staphylococcus aureus in both children and adults. The Staphylococcus aureus is transmitted to nares by contaminated hands and from surfaces where it can survive for months. Colonized health care worker acts as a reservoir for the spread of Staphylococcus aureus to uncolonized susceptible patients. The study aims to investigate the nasal carriage of Staphylococcus aureus among health care workers.

Methods & Materials: A total of 192 nasal swabs were obtained from health care workers (nursing staff, attendants, and laboratory personnel) and cultured for carriage of Staphylococcus aureus. Isolates were identified based on their growth on mannitol salt agar, blood agar, gram staining, catalase and tube coagulase tests. All the isolates were subjected to in vitro antibiotic susceptibility testing as per CLSI guidelines.

Results: Among 192 health care workers, 28 (14.58%) were Staphylococcus aureus carriers. Highest rate of Staphylococcus aureus carriage was seen among central clinical laboratory staff (30.77%) followed this in orthopaedic ward (23.08%). Among 28 Staphylococcus aureus isolates 4(2.08%) were MRSA, 1(0.52%) inducible clindamycin resistant, 1(0.52%) low level mupirocin resistant. None of the isolate had constitutive clindamycin resistance and high level mupirocin resistance. The isolates were sensitive to Vancomycin (100%), Rifampicin (100%), Clindamycin (96.43%), Erythromycin (75%), ciprofloxacin (71.43%), Cotrimixazole (50%), Penicillin (0%).

Conclusion: It is important to develop proper protocol for eradication of nasal carriage and strict hospital infection control policy to reduce carriage of Staphylococcus aureus among health care workers.
Evaluating the effect of hand washing and sanitization on the microbial burden of the hand
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Background: Most communicable diseases are transmitted by hand; from nosocomial to community acquired diseases. Consequently, hand washing and the use of sanitizers are advocated in hospitals, eateries and public places where contact by hand is frequent. The effects of hand washing and use of sanitizer alone and in combination in reducing the microbial load of the hand in line with specified hand washing guidelines were determined.

Methods & Materials: Five hundred students of Covenant University were enrolled in this study and divided into three different groups; hand washed, hand sanitized and hand washed and sanitized. The roles of keeping long nails and toilet hygiene after defecation (use of tissue paper versus water to clean the anal region) on the microbial load and types of hands were determined. Samples were collected with moistened swabs before and after each hand treatment, and then cultured for both qualitative and quantitative microbial assessment. A selected group was followed up to determine the effect of improving toilet hygiene on the microbial load and type of the hands. To evaluate the impact of proper and frequent hand washing, swabs from door handles of classrooms, offices and toilets were cultured.

Results: Application of sanitizers on unwashed hands did not significantly reduce the microbial burden especially in hands with Pseudomonas, Klebsiella and Staphylococcus species. Application of sanitizer after hands were washed gave a 2-4 log10 CFU microbial reduction in 40% of cases while in 55% cases reduction in count was as high as 6-7 log10 CFU. Long nails and the use of water to clean the anal region after defecation accounted for the build up of hand microflora (p > 0.05). Hands were re-colonized with same flora and at approximately same population within one week of decolonizing. Microorganisms from door handles irrespective of location were qualitatively the same as those isolated from the hands and include staphylococci, streptococci, diphteroides, pseudomonas, klebsiella, escherichia, enterobacter, enterococci, bacillus, candida and moulds.

Conclusion: The multiple factors associated with re-colonization of decolonized hands pose the question as to how frequent should hands be washed?
Study of Listeria monocytogenes contamination in raw milk and some Moroccan traditional dairy derivatives (Lben and Jben)

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**Background:** Listeria monocytogenes is the causative agent of Listeriosis, a serious food-borne disease primarily associated with the consumption of processed foods that require no further cooking by the consumer. Milk and dairy products have been implicated. The objective of this study was to determine the incidence of *Listeria monocytogenes* in raw milk and its two traditional drives, the “Lben” (traditionally fermented skimmed milk) and “Jben” (traditional soft white cheese) commercialized in Fez city situated in the center northern Morocco throughout a year.

**Methods & Materials:** A total of 288 samples of three dairy products were collected from eight dairies traditional belonging to various sectors of Fez city throughout a year. Isolation and identification of *Listeria monocytogenes* were carried out according to Moroccan standards NM08.0.110 (2004). Selected physicochemical parameters were also carried out in parallel. The *L. monocytogenes* strains that were isolated in this study were tested against eleven antimicrobial discs.

**Results:** The overall incidence of *Listeria monocytogenes* contamination was 17.70%. It was present in the three dairy products. The results of this study revealed also a variation of contamination from one sector to another with a higher incidence of contamination in milk and dairy product samples collected in the autumn and winter, suggesting a link between management practices feed, poor hygienic conditions and *Listeria monocytogenes* contamination. The physicochemical results show an acidic pH in the “Lben” and “Jben” that in raw milk, indicating a significant lactic fermentation of these two products.

**Conclusion:** The levels of contamination found justify the control of the feeding cattle, milk pasteurization and enforced the general principles of food hygiene in order to reduce consumer’s exposure to *Listeria monocytogenes*. 
Clinical symptomatology and treatment with ambisome in cases with visceral leishmaniasis hospitalised in pediatric infective care unit, Tirana, Albania

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Background: This is a descriptive study to present clinical data and treatment with ambisome in children with visceral leishmaniasis, hospitalised in Pediatric Infective Care Unit, during the timeperiod january 2011-september 2015.

Methods & Materials: In this study are involved 148 children with visceral leishmaniasis , aged 1-month-14 years old hospitalised in this Unit. Except the clinical symptomatology, there is also the interpretation of the two treatment groups, 58 cases with ambisome and 90 with glucantime.

Results: Clinical characteristics include fever( 98.65 %), reduced appetite(39.9%), pallor(100%), weight loss(98%), nausea(14.9%), diarrhea(35.1%), coagulation disorders(12.2%), abdominal distension(45.3%), edema(28.4%), ikter(3.4%), hepatosplenomegaly(100%), inceased lymph nodes(10.8%).
58 children (39.2 cases) were treated with ambisome according to the respective protocol, from the 1-st to 5-th day, on the14-th day and 21th day, 3mg/kg/day, iv. The temperature was normalised after 2 days, clinical symptomatology after 5 days, pancytopenia after 5 days, splenomegaly after 3 weeks and the average of hospitalisation was 8.32 days.

In comparision with the above mentioned medication, glucantime treatment , in 90 children (60.8 % cases), was used for 28 days, 75mg/kg/day im. The temperature was normalised after 7 days, pancytopenia after 2 weeks, splenomegaly after 1 month and the average hospitalization was 31 days.

Conclusion: From the results of our study, we conclude that therapy with ambisome has priority in relation to glucantime, not only regarding the comodity of taking the medicine and timeperiod, but also in the faster progress of clinical and laboratoric indexes.
Non-microbiological system to improve hospital hygiene in a critical care unit (CCU)

Hospital Alemán, Buenos Aires, Argentina

**Background:** It is known that patient’s flora contaminates the environment favoring microorganism’s dissemination. Hospital Hygiene is therefore critical as an infection control measure. Aiming to improve it we decided to measure it and give feedback to the cleaning staff in order to stimulate them. We also tried to assess if effectiveness gained was maintained through time.

**Methods & Materials:** Prospective before-after study in the 30-bed CCU of a private hospital in Buenos Aires, Argentina (Ten intensive care beds, 10 intermediate care beds and 10 coronary care beds).

- **Pre-intervention** (3 months): With an invisible-ink pen we made ten marks in different surfaces of each room and controlled if they persisted 24 hours later. We calculated percentage of marks vanished.
- **Intervention:** We showed the results to the cleaning-staff and reviewed the right technique with them.
- **Post-intervention** (3 months): During the first 3 months, we evaluated the hygiene monthly and informed the results obtained. The cleaning staff was asked to complete a satisfaction survey.

**Results:** Cleaning efficacy improved statistically significant after the intervention

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The less cleaned components were the infusion pumps (84%) and the ends of the beds (87%).

The 84% of the survey-responders thought cleaning had improved and 79% perceived their work was more appreciated.

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Continuous controls are required to sustain achievements through time.

**Conclusion:** To retrieve results is a beneficial strategy to improve cleaning. To analyze data together with the staff allows finding out real and specific goals. Control is essential to sustain results through time.
Attitude is a little thing which makes a big difference – KAP on isolation practices amongst visitors

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**Background:** The impact of contact isolation on patients and health care workers (HCW) has been reviewed in several studies with varying results. There is limited literature from India on Knowledge attitude and practice (KAP) of isolation precautions on visitors of patients in contact isolation. The aim of our study was to survey visitors to understand their (KAP) regarding the same.

**Methods & Materials:** The study was carried out at a tertiary care oncology centre in south India where infection control policy includes contact isolation for all patients either colonized or infected with *methicillin-resistant Staphylococcus aureus* (MRSA), *Vancomycin resistant enterococcus* (VRE), *multidrug-resistant gram negative organism* (MDRO), and *Clostridium difficile* infection. Survey questionnaires were distributed to the visitors of patients in contact isolation during the period of March 2015 to June 2015. The filled questionnaire was collected back. An audit on the practices was undertaken by an ID doctor or a nurse and was documented.

**Results:** A total of 31 attendants interviewed. Most respondents were in the age group of 30-40 years (61.3%) with an education level of graduation and above (61.3%). Ninety percent of the patient’s visitors were aware regarding their patient being in contact isolation, with the nursing staff being their most common source of information (83.9%). A total of 70.97% of respondents were aware about appropriate method of contact isolation (gloves, gown and hand hygiene). Ninety percent of the respondents agreed regarding the importance of contact isolation in prevention of spread of infection, only 54.8% found it minimally inconvenient. Majority (74.2%) of the respondents were satisfied with the information provided on contact isolation and majority felt that education by nursing staff (77.4%) is the most effective way of conveying information rather than the sign boards (3.2%). Majority of visitors (74.2%) were practising appropriate contact isolation methods (Gloves, Gown and Hand Hygiene).

**Conclusion:** Based on our study results, visitors seem to understand the purpose of contact isolation in the inpatient setting. We found that the visitors had an overall positive perception about contact isolation although it was considered inconvenient. They were often educated by nurses, which appears had the greatest impact with good adherence rate.
Surveillance of nosocomial Clostridium difficile infection (nCDI) in a large tertiary community hospital
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**Background:** Clostridium (C.) difficile infection (CDI) is of rising concern globally as an important nosocomial infection. Apart from higher incidences the increasing morbidity and mortality by virulent strains causes problems. Bundle approaches including hygiene measures as well as antibiotic stewardship policies are used to reduce the burden of CDI.

**Methods & Materials:** A structured surveillance of nosocomial CDI (nCDI, defined by diarrhea occurring >72 hours after admission with subsequent detection of C. difficile by direct Toxin assays or toxinogenic culture) was established to explore individual and environmental risk factors for nCDI compared to non-nosocomial CDI episodes. Patient data as well as ward data (antibiotic consumption, glove and disinfectant use, numbers of hygiene events and compliance with standard cleaning protocols) were assessed and compared for the occurrence of nCDI on an individual patient and ward level.

**Results:** All primary CDI episodes in 2014 (n=168) were included. In 105/168 episodes the definition for nCDI was met. Comparing patients with nCDI or non-nosocomial CDI those with nCDI had more often surgical interventions, more antibiotic exposure (all p<0.05). Age, gender, use of medications other than antibiotics and history of CDI were not associated with nCDI. Wards with high nCDI incidences had lower closing disinfection frequencies for rooms after dismission of CDI patients, more use of aldehydic disinfectants compared to sporicidal substances in daily ward cleaning (all p<0.05) compared to those with lower nCDI incidence. Additionally, more overall antibiotic consumption and less use of tetracyclines (all p<0.01) were also associated with high nCDI incidences. No differences were found regarding to hand hygiene, glove use or all hygiene events on a daily basis or per 1000 patients.

**Conclusion:** In this setting, antibiotic consumption density on the respective wards as well as individual antibiotic exposures were the most imminent risk factors for nCDI. Adherence and compliance to ward cleaning protocols as well as the selection of the disinfectant used was also important. Hand hygiene frequencies or glove use densities were of less importance regarding to the risk of high nCDI incidences.
Brucella exposure in a microbiology laboratory in South India - Never sniff a gift fish

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**Background:** Brucellosis is one of the most widely reported laboratory acquired bacterial infections. Microbiology laboratory workers are at increased risk of brucellosis through unsuspected exposure to cultures from clinical specimens. Brucellosis is common in India, but no such laboratory exposure was reported in the Indian literature. Here we report our experience in managing an exposure to *Brucella melitensis* culture in a microbiology laboratory.

**Methods & Materials:** In January 2015, a 10 year old boy admitted with the diagnosis of septic arthritis in a tertiary care hospital in South India. The aerobic blood culture was processed in biosafety level II microbiology laboratory of the hospital grew *Brucella melitensis*. Before the identification of Brucella, the microbiology laboratory personnel present in the laboratory were exposed. Emergency control measures (risk assessment, post exposure antibiotic prophylaxis, symptomatic monitoring & serological testing) as per CDC guidelines was imitated to prevent an outbreak of laboratory associated Brucella.

**Results:** Totally 13 microbiology laboratory personnel were present during the processing time of Brucella culture. Their exposure level and outcome was discussed in table -1.

<table>
<thead>
<tr>
<th></th>
<th>High Risk (Sniffed the culture plates)</th>
<th>No Risk (Present within the Microbiology Lab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of Microbiology Lab Personnel</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>3 week Post Exposure Antibiotic Prophylaxis given</td>
<td>Yes for all five personnel (all 5 completed the course)</td>
<td>No</td>
</tr>
<tr>
<td>Monthly symptomatic Screening for 6 months</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Baseline Serological testing</td>
<td>Done (all negative)</td>
<td>Not done</td>
</tr>
<tr>
<td>6 months follow up testing</td>
<td>Done (all negative)</td>
<td>Not done</td>
</tr>
</tbody>
</table>

**Conclusion:** The immediate notification of the exposure and emergency measures prevented the laboratory associated outbreak of Brucella in our institution. However, Laboratories in non-endemic areas must prepare for potential isolation of Brucella species and periodic education to laboratory staff about handling the specimens may prevent such exposures in the future.
Reflection on observation: A qualitative study using practice development methods to explore the experience of being a hand hygiene auditor in Australia

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Background: The Australian Public Health Care system has adopted an observation model for auditing hand hygiene practice in healthcare workers. The data gathered is used as a healthcare service performance indicator and is publicly available. This qualitative study used Practice Development methods, in particular a values clarification tool, in order to gain an understanding of the experiences of hand hygiene auditors. These methods were also used to identify the enablers and barriers to the successful carriage of the auditors’ role from their perspective. The intent of this study was for the results to inform the development of a strategy to support both the individual auditors, and the local sustainability of the hand hygiene auditing programme.

Methods & Materials: The methodology employed qualitative interpretation of focus group discussions involving healthcare workers trained as hand hygiene auditors working in nine regionally-based public hospitals and associated community-based services in New South Wales (NSW), Australia.

Results: Twenty-five participants identified congruous themes of the need for peer and managerial support, improved communication and feedback, and consideration for succession planning. There was consistency amongst participants’ identified most significant barriers in undertaking the role. These findings add support to what is already known in terms of “time and resources” adding new insights into cultural issues.

Conclusion: Importantly this study provides evidence of the need to support individual hand hygiene auditors, or indeed any auditor in healthcare who has a clinical load, in order to sustain the programme beyond the training period. This research has provided an overview of the enablers required to be in place for such a programme to be a success. This is of significance as this model can be translated across any audit programme requiring observational data collection. This research will be of interest nationally and globally as there is little published on the lived experience of hand hygiene auditors.
Enterococcus spp synergises the antimicrobial activities of conventional antibiotics against ciprofloxacin-resistant Salmonella enterica serovar Typhi

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**Background:** *Salmonella enterica* ser. Typhi is the causative agent of the clinical condition called typhoid fever that results after an incubation period of 10 to 15 days following infection. The main line of treatment of typhoid fever includes antibiotics such as fluoroquinolones and aminoglycosides; however, several studies have reported decreased susceptibilities of fluoroquinolones among *Salmonella* spp isolated from human infections. Thus, this necessitates the studies to explore alternative or adjunct therapeutic agents.

**Methods & Materials:** In this study we isolated and screened the antimicrobial potential of 92 vaginal lactic acid bacteria from healthy women against *S. enterica* ser. Typhi MTCC 733 by using agar gel diffusion assay. Further, the susceptibility of *S. enterica* to various antibiotics and the synergistic activity of the culture supernatant (CS) of the isolate 12a along with antibiotics was determined by using Kirby Bauer disk diffusion and chequerboard titration methods, respectively.

**Results:** The isolate no. 12a, identified as *Enterococcus* spp. by using physico-chemical characterisation showed broad spectrum antimicrobial activity against many Gram-negative pathogens including *S. enterica* MTCC733. The antimicrobial activity of the CS of 12a was proteinaceous in nature and lost its activity on treatment with pepsin, proteinase K and papain. The minimum inhibitory concentration of the CS was 2133 AU/ml and was stable over a wide pH range of 3-11. Further, the antimicrobial activity of the CS was lost at 100°C; 1 hr treatment. The susceptibility of *S. enterica* to various antibiotics was determined and it showed reduced susceptibilities to many fluoroquinolones and aminoglycosides. Chequerboard titration assay showed that CS of 12a synergised antimicrobial activities of the antibiotics belonging to the classes fluoroquinolones, aminoglycosides and β-lactam against *S. enterica*.

**Conclusion:** In conclusion, the study indicates the potential of the probiotic strains of enterococci as adjunct therapeutic agent against resistant forms of *S. enterica*.
Antiviral effect of Glycine coated Iron oxide nanoparticles iron against H1N1 influenza A virus

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Background: Influenza virus is a common human pathogen that has caused serious respiratory illness and death over the past century. It always had potential to cause widespread pandemics whenever a new type of Influenza strain appeared in the human population and then spread easily from person to person. Although the treatment of influenza produces rapid suppression of H1N1 influenza A virus infection but this effect is not often sustainable due to the emergence of drug-resistant H1N1 strains. Therefore, it is important to develop new antiviral strategies to combat wild-type and mutant H1N1 influenza A virus infections. Therefore nanotechnology based antiviral therapy has been developed, modifications of existing antiviral compounds and development of novel antiviral iron oxide is a prime area of research

Methods & Materials: Glycine coated iron oxide nanoparticles with particle size in the range of 10-15 nm anti-influenza activity was elucidated (in vitro and in vivo) utilizing pandemic influenza strain A/H1N1/Eastern India/66/pdm09 (H1N1-pdm09). Anti-influenza activity was measured by plaque inhibition and quantifying viral transcripts using quantitative real-time PCR following treatment with Iron oxide nanoparticles in a dose- and time-dependent manner. Cell viability of Iron oxide on MA104 cells was assessed by MTT assay the antiviral activity of the iron oxide nanoparticles was evaluated by comparing the TD₅₀ ratio of viral suspensions treated with the composites to untreated suspensions

Results: 50% cell viability (TD₅₀) was observed at 4.25 pg±2pg of Iron oxide nanoparticles during in vitro study. The percentage of plaque inhibition determined for each drug concentration, the IC₅₀ (50% virus reduction) of H1N1-pdm09 strain (0.5 moi) in vitro in MA104 cells by the pfu method was observed at 0.1pg after 72 h. The antiviral activity determined by change in viral RNA transcripts within 24 h of virus infection by RT-PCR, 08 fold reductions in virus found when treated with Iron oxide nanoparticles.

Conclusion: Stable Fe₃O₄ nanoparticles were successfully synthesized. In vitro assay showed that at the highest dose of iron oxide (6.5pg/mL), the growth of H1N1 virus was inhibited significantly compared with the control samples, Indicates that Iron oxide nanoparticles have potential for use as antiviral activity.
Design of a study to examine contact mixing and acute respiratory infection in Ballabgarh, Haryana

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Background: Data on contact mixing are critical to understanding the spread of epidemics and pandemics that may disproportionately affect developing countries, but few studies have estimated contact mixing in these settings. We describe the design of a planned contact mixing study nested within an ongoing acute respiratory infection (ARI) study in Ballabgarh, Haryana. The contact mixing study aims to 1) describe the social contact patterns of individuals in this rural Indian population, where caste, gender, and age hierarchies are hypothesized to influence interactions, and 2) examine the impact of contact heterogeneities on influenza and general ARI risk after controlling for age.

Methods & Materials: Along with weekly household visits to capture ARI and influenza episodes in all residents in a sample of 900 households, we will capture information on social contacts over a sampled day from all individuals in these households. A structured questionnaire of social contacts (conversational within 3 feet or physical) over the past 24 hours will be administered in a face-to-face interview with each respondent. Respondents will report age and sex of contacts, along with the total duration of encounter(s), place of contact (at home, work, school, during transport, or other), and location of the contact of maximum duration (geocoded).

Results: In a pilot study conducted in July 2015 that served to establish feasibility, 77 individuals reported 922 contacts during the previous 24 hours. Assortative mixing (mixing with similar people) by age and sex was apparent. Females made fewer contacts than males (one-way ANOVA $F(1, 75) = 4.89; p=0.030$) and had more contacts within the home than outside compared with men ($F(1, 75) = 5.42; p=0.023$). We will present analyses from the planned study, including age contact matrices, and draw preliminary conclusions on mixing in households and other locations in this rural Indian population.

Conclusion: One limitation of our study is that the validity of self-reported contacts may vary by age and gender. This novel study in India will, however, lay the foundation to explore social mixing patterns using passive and technological data collection methods, as well as for mathematical and computational explorations of influenza transmission and interventions to reduce disease burden.
Social media for infection control and prevention
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Background: India is a vast country with diversities and various infectious diseases. Large number of Indians uses social media.

Objectives: To elicit the view of college students on their participation for infection control and prevention.

Methods & Materials: A questionnaire survey was circulated among 200 college students to elicit their willingness to learn and support infection control and prevention through Social media. The questionnaire consisted of willingness to learn on disease outbreaks, symptoms, and health care advice; report to authorities and participate in prevention aspects.

Training on selected aspects of infection among all students of higher education was checked through respective web sites.

The data was analysed statistically.

Results: Of the 200, 180 were familiar with social media and were willing to participate on all aspects of infection control and prevention. They were also willing to pass on the infection related information to others nearby and far away through social networking and support the governmental programmes for prevention. There were no structured training programmes on selected aspects of infection among all students of higher education.

Conclusion: College students are interested in infections and infection control, and in social media. Hence, every student shall be informed and empowered on basics and prevention aspects of infectious diseases through National Social Services, Youth Red cross or other several social service systems prevalent in respective colleges, in an uniform manner and monitored by University Grand Commission (UGC), as infection related aspects do not receive due attention. For effective control and prevention of infection in India, activities and participation of students and colleges on infection control have to be incorporated in the assessment of the college by various Accreditation councils or Assessment systems. Accordingly Health and Family Welfare Department of India should use Social Media to empower college students, and utilize their services for infection control and community education.
Institutional Chickenpox Prevention Programme (ICPP) in a tertiary care hospital in Singapore: Lessons from epidemiology and contact tracing

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Background: Chickenpox vaccination in Singapore is not mandatory. At the National University Hospital (NUH), nosocomial transmission has led to a sentinel event and secondary cases. To prevent future transmissions, we studied the impact of Institutional Chickenpox Prevention Program at NUH.

Methods & Materials: NUH is a 1000 bedded tertiary care hospital in Singapore, with negative pressure isolation capability in 179 rooms and staff strength of approximately 7300. A retrospective audit of contact tracing data was done from January 2010 to June 2014, with probabilistic modeling to predict costs and number of future varicella infections. Data was obtained from clinical records, hospital information systems and the human resource department.

Results: There were 51 cases of chickenpox including 15 staff (Average 11.3 cases per year in total, 3.3 per year among staff). One index resulted in secondary transmission. The median number of staff contacts per index case was 4 (IQR 2-13) with 0 (IQR 0-2) being non-immune staff contacts. Direct costs and man hours lost in high risk areas (obstetrics and oncology), were significantly higher. Current vaccination strategy A, where staff with negative or uncertain history of prior chickenpox, are screened with serum Varicella zoster virus immunoglobulin (VZV IgG) levels was compared with two scenarios B and C using probabilistic modeling, (B: VZV IgG for all existing and new staff; C: VZV IgG for existing staff with negative history and all new staff). After 10 years, expected number of chickenpox infections per year are 3, 1, and 2 under Strategies A, B and C respectively. Number of susceptible healthcare workers is 744.6 for A, 109.5 for B and 355.5 for C. Cumulative costs for Strategies B (599048 SGD) and C (496752 SGD) are 65% and 37% higher as compared to Strategy A.

Conclusion: Chickenpox adds significant burden in terms of costs and man hours lost. Current strategy relies on history and contact tracing, to keep the number of infections at 3 per year. Wider screening strategies incur greater cost, but targeted interventions such as laboratory screening for international staff and those working in high risk wards may be more cost effective.
Cultural rationale and architectural designs of Isolation Centres (ICs): A case of dangerous pathogens such as Ebola

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Makerere University, Kampala, Uganda

Background: Socio-cultural and Architectural suitability of a medical facility are very key to infection prevention and control. ICs should accommodate the well-being of the users. From the recent Ebola outbreak in West Africa, culture played a significant role to the spread of the disease. ICs should be designed cognisant of the risks of spread of disease such as Ebola through socio-cultural practices and also architectural unsuitability. Therefore the space designed for this function should be well thought through to achieve the two contradictory statements. When ICs are poorly designed, they may cause more harm than good because they may lead to infection of even the medical workers and surrounding communities. They are also completely useless and a complete waste of the resources. The objectives of the study are; 1. To examine the socio-cultural and architectural suitability of ICs. 2. To design ICs that are socio-cultural and architecturally suitable. 3. To establish factors that hinder socio-cultural and architectural suitability.

Methods & Materials: The research covered the ICs at Entebbe the country’s main entry point and Kampala the country’s capital city, Uganda. This is because infectious diseases normally spread from country to country and also we have seen the impact of such infection on capital cities unlike the usual that is normally in rural areas. I carried out interviews and discussions with key persons. I did desk study of the drawings, I physically visited the centres and also took part in VHF and EDPs trainings.

Results: The existing isolation centres and medical facilities are not socio-culturally and architecturally adequate thus a risk to infection prevention and control. They are also unfriendly to the users thus resistances for staff and also for patients to be taken there.

Conclusion: There is need for a socio-culturally and architecturally suitable IC at the country’s main international access point and the capital city. Infectious disease units should be created in all national hospitals and should be well designed. I made designed for both permanent and temporarily ICs that are socio-cultural and architecturally suitable.
Modifying the existing water tap system to create a no touch, cost effective solution

G. Nakibaala
Makerere University, Kampala, Uganda

**Background:** Infection prevention and control is very key in health care. With the main component being the hand hygiene. Mid-1800s Ignax P. Semmelweis established that hospital-acquired infections (HAI) were transmitted via health care workers' (HCW) hands. Contaminated HCW’s hands are the commonest route of HAI transmission. Nosocomial spread (patient to health care worker) in the health care setting is key in amplifying the infectious diseases outbreaks. Research shows that a hospital has a lot of infections, hand hygiene contributes about 60% reduction in infection spread. The most effective and cost efficient way to prevent the spread of germs/infections is by using soap and water. From my analysis, hand hygiene facilities like sinks and sanitisers placed all over the hospitals have been about 40% successful since people fear to get infections from them through touch.

**Methods & Materials:** I took a case of referral hospitals in Kampala, Uganda and health centre IVs including the national referral hospital. I physically visited the centres and had interviews and discussions with the key persons including persons from ministry of health.

**Results:** Some medical facilities have washing facilities, they have constant water supply and soap. According to Infectious Disease Institute, 40% of health care workers practice proper hand hygiene. Both health care workers and patients do not want to use the tap. Research has been done and solutions put in place but they are very expensive thus ineffective in low and medium income countries. These include sensors--we have tried to adopt but the initial cost and maintenance has proved expensive. Also use of tissue is highly costly and medical personnel tend to forget using it. Even the routine rinsing of the tap after use can easily be forgotten.

**Conclusion: No hand contact** - Only contact with device is through a foot-pedal, no fear of infection, reducing the possibility of human error

**Lower water consumption** - water only runs when the pedal is pressed

**Cheap - US$3** Purchase price, low production cost, lower maintenance cost and no external power required

**No extra installation costs** - no cost of demolishing and creating a completely new system, universal fitting
Investigation of an outbreak due to Serratia marcescens in a neonatal intensive care unit in a tertiary care hospital

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Background: *Serratia marcescens* (*S. marcescens*) is an accepted clinical pathogen, particularly in high risk settings. Numerous outbreaks have been noted particularly as bloodstream infections in NICU. It is difficult to treat because of the resistance to antibiotics including beta-lactams and aminoglycosides. We describe the epidemiological features of *S. marcescens* infection outbreak in a 20 bedded tertiary care NICU.

Methods & Materials: In April, 2015 we had a low birth weight baby with septicaemia due to *S. marcescens*. It was sensitive to all antibiotics except colistin and polymixin B. The baby received cefotaxime and gentamicin but died. After a week, within a period of 9 days 6 neonates, admitted due to other reasons [3 with hypoxic ischemic encephalopathy, 2 with very low birth weight (VLBW), 1 with meconium aspiration syndrome] were having *S. marcescens* sepsis. The organism was diagnosed by blood culture in Bactec 9050 system with standard protocol and sensitivity was performed according to CLSI guidelines. Promptly several environmental samples, hand swabs, iv fluid samples and rectal and oral swabs of other neonates were processed. Affected neonates were isolated and dedicated nursing staffs were allotted for them. Proper hand washing and diaper disposal were strictly emphasized.

Results: *S. marcescens* was obtained from a running IV fluid bottle of an unaffected neonate and from a normal saline bottle which was being used for reconstituting IV fluids for the neonates. Two VLBW neonates were found to be colonised with the same strain. One of them eventually developed sepsis with ventilator associated pneumonia with the same strain being isolated from blood and bronchoalveolar lavage fluid. No other samples revealed the organism. These subsequent cases and colonisers were treated aggressively with IV meropenem with success. After the reinforcement of the infection control measures, the outbreak was brought under control.

Conclusion: The results indicate the importance of active surveillance for the prompt detection of an outbreak by Infection Control Committee so that reinforcement of infection control measures could be taken to decrease the morbidity/mortality due to opportunistic pathogens in hospital settings.
Disinfection against healthcare-associated infections: Current status and recent progress in products and procedures  
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**Background:** Healthcare-associated infections (HAI) continue to be a major global threat. Indeed, many ongoing societal changes and rampant antimicrobial resistance are adding further to their health and economic impacts. With dwindling options for chemotherapy and limited availability of safe and effective vaccines, there is increasing reliance on disinfectants for infection prevention and control (IPAC).

**Methods & Materials:** However, the selection/use of disinfectants in itself is a challenge with various blends of some 275 different types of actives in thousands of products with varying label claims, safety profiles and cost structures. Further, a closer scrutiny reveals disparities between their pre-market testing and actual field-use in healthcare and other settings. There are also mounting concerns on the human and environmental safety of certain widely used disinfectant chemicals, label directions as well as the listing of numerous irrelevant pathogens on product labels. In many healthcare settings purchasing decisions are generally based on cost alone, and the front-line environmental services staff are often inadequately trained and monitored for their crucial roles in environmental decontamination, frequently resulting in suboptimal disinfection practices.

**Results:** All these factors together undermine the potential benefits of disinfectant use. This presentation will critically review the above-mentioned issues with emphasis on disinfection of high-touch environmental surfaces (HITES) and highlight recent developments in quantitative and internationally-harmonized test protocols to assess such formulations against major classes of human pathogens and also list criteria for choosing and applying disinfectants for optimal effectiveness and safety. The information to be presented should: (a) better inform end-users on prudent selection and application of disinfectants for effectiveness as well as workplace and environmental safety, (b) convince materials managers to consider the overall benefits of disinfectant use and not the cost alone, (c) encourage manufacturers to explore innovative and more effective ways of countering HAI, (d) urge regulators to update the requirements and procedures for pre-market registration of disinfectants, and (e) assist infection preventionist in lobbying for appropriate resources for better IPAC via environmental hygiene.

**Conclusion:** Proper and regular disinfection of HITES in particular must be an important adjunct to hand hygiene in order to successfully interrupt the spread of HAI for patient and occupational safety.
Decontamination of high-touch environmental surfaces in healthcare: Quantitative assessment of disinfectant pre-soaked wipes.

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**Background:** High-touch environmental surfaces (HITES) are increasingly recognized as vehicles for healthcare-associated pathogens. While such surfaces are normally decontaminated by wiping with disinfectant-presoaked towelettes (DPTs), DPTs are rarely tested simulating their field use. Also, the label claims of environmental surface disinfectants seldom include the wiping action. This study used a new device (The Wiperator) to assess three commercial DPTs for decontaminating HITES and to transfer the acquired contamination to clean surfaces.

**Methods & Materials:** Each disk (1 cm diam.; 0.7 mm thick) of magnetized and brushed stainless steel received 10 µL (~10^7 CFU) of *Staphylococcus aureus* or *Acinetobacter baumannii* in 0.3% bovine serum albumin and dried. The DPTs and a control fabric (J-Cloth wetted with Tryptone-saline) were tested by an orbital motion for 5 or 10 s at a pressure of 150 g. Each disk was eluted in 1.0 mL of an eluent/neutralizer, the eluates assayed, and log_{10} CFU reduced or transferred calculated. The product performance criterion was >4 log_{10} (>99.99%) reduction in CFU of both organisms by wiping with no detectable CFU transferred.

**Results:** J-Cloth removed *S. aureus* and *A. baumannii* by 2.88 log_{10} and 3.23 log_{10}, and transferred 4.13 log_{10} and 4.06 log_{10}, respectively. In all tests, DPTs with 0.5% accelerated H_2O_2 reduced the CFU of both the bacteria to undetectable levels with no detectable transfer. The product with a mixture of quaternary ammoniums (quats) and ≤24% ethanol reduced the *S. aureus* and *A. baumannii* CFU by 5.53 log_{10} and 5.80 log_{10}, respectively; the corresponding transfers were 2.82 log_{10} and 2.04 log_{10}. The product with a mix of polymeric biguanide and two types of quats reduced the *S. aureus* and *A. baumannii* CFU by 4.71 log_{10} and 5.73 log_{10}, respectively; the transfer for *S. aureus* was 2.5 log_{10} but no detectable transfer for *A. baumannii*.

**Conclusion:** The device and the protocol described can quantitatively determine HITES decontamination as well as transfer of the acquired microbial contamination on DPTs to clean surfaces for better risk assessment and in making more relevant and reliable claims on marketed DPTs.
Dynamical behavior of SIRS epidemic model with media awareness as control strategy

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**Background:** In the modern era, the spread of disease is very fast with the transportation allowing more than a million people a day to cross international borders. To control this spreading of disease, the health officials may have various pharmaceutical and non-pharmaceutical (wearing masks, closing schools, isolation, staying at home etc.) options. Media plays a very important role to communicate awareness in public for use of non-pharmaceutical interventions (NPIs) to control the epidemics.

**Methods & Materials:** Determine the basic reproduction number by using a next generation matrix operator. Discuss stability criterion of the equilibrium points of the model with bifurcation theory. Carry out parameter sensitivity analysis by using the normalized forward sensitivity index. Perform the numerical simulation of the model to verify the results of qualitative analysis using MATLAB.

**Results:**

Result 1. The disease free equilibrium (DFE) is locally asymptotically stable, if $R_0<1$ and unstable, if $R_0>1$.

Result 2. The endemic equilibrium (EE) is locally asymptotically stable for $R_0>1$, but close to 1.

Result 3. The coefficient of media awareness $m$ does not effect $R_0$.

Result 4. The level of endemic equilibrium is significantly affected by media coefficient $m$.

**Conclusion:** In this paper, we proposed a SIRS epidemic model incorporating media awareness as control strategy and investigated the asymptotic stability of the model in both disease-free equilibrium and endemic equilibrium states. The disease free equilibrium is locally asymptotically stable for basic reproduction number $R_0 < 1$, a transcritical bifurcation occurs at $R_0 = 1$ and a unique locally asymptotically stable endemic equilibrium exists for $R_0 > 1$. We observed that the coefficient of media awareness $m$ does not effect $R_0$ and hence the qualitative features of the model remain unaltered, but the level of endemic equilibrium is significantly affected by media coefficient. We calculate normalized forward sensitivity indices for the basic reproduction number and state variables at endemic equilibrium with respect to various parameters and identified respective sensitive parameters. Numerical simulations of the system justify the analytic findings and we also observed that the level of endemic equilibrium is significantly affected by media coefficient $m$. 
Need for more communication between hospitals in different countries: Two cases of carbapenem-resistant Enterobacteriaceae
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Background: Carbapenem-Resistant Enterobacteriaceae (CRE) are a significant global public health burden. We propose to describe two cases of CRE containing blaNDM resistance and share lessons learned.

Methods & Materials: At Parkland Memorial Hospital, a 770-bed public academic hospital in Dallas, Texas, USA, less than one patient per month is diagnosed with CRE. For surveillance, definitions from the Centers for Disease Control and Prevention are used. Active screening cultures are not performed routinely. Carbapenem non-susceptible Enterobacteriaceae isolates are flagged to the infection preventionists via real-time email alerts and further tested via Hodge test and/or PCR testing for specific carbapenemase genes. Hospitalized patients are placed in enhanced contact isolation precautions. Roommates and other patients who were potentially exposed before placement in isolation precautions are actively screened for presence of CRE using oropharyngeal and rectal swab cultures.

Results: Thirty-two cases of CRE have been identified since 2009. The bacteria represented are Enterobacter cloaceae (14), Klebsiella pneumoniae (7), Citrobacter freundii (2), Enterobacter aerogenes (4), Escherichia coli (4) and Serratia marascens (1). Of the 32 cases, 29 were associated with healthcare, including 19 hospital-onset cases. The two cases with blaNDM type resistance are the first of their kind at Parkland. Case 1: A 63 year-old South Asian female with diabetes and end-stage kidney disease presented with uremic symptoms. Blood cultures were positive for Klebsiella pneumoniae. She was diagnosed with mitral valve endocarditis and she was treated with antimicrobial therapy and hemodialysis. Cultures of her oropharynx and rectum were negative and therefore further contact tracing was not done. Case 2: A 31 year old South Asian female had a lap sleeve gastrectomy in India 1 month prior to admission. She presented with flank pain and was diagnosed with nephrolithiasis and Escherichia coli urinary tract infection. Two patients who shared a semi-private room and a bathroom with her before diagnosis were screened and found to be free of colonization in the oropharynx and rectum.

Conclusion: International inter-hospital sharing of any available infection control information might have assisted with earlier placement in isolation precautions and better surveillance and feedback. Infrastructures to facilitate sharing of such information are needed.
Relation of risk factors and mortality in the Carbapenem-resistant Klebsiella pneumoniae infection: Case control study
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Ondokuz Mayis University School of Medicine, samsun, Turkey

**Background:** Epidemiological importance of Carbapenem-resistant Klebsiella pneumoniae (CRKP) is to be a nosocomial pathogen, which has come to be known for the last 10 years in our country and for 20 years in the world. Infections developing with CRKP has been threatening the community health care due to limited treatment options and high mortality rates in despite of the whole improvements in the field of medicine at the present time.

**Methods & Materials:** Our study was carried out between the dates January 2010- September 2014. Patients, who were hospitalized at least for 72 hours in the hospital, are 18 years old or older, have CRKP growth and are admitted as active and are given treatment, were included in the study. In the same period patients, who hospitalized in the same ward and have not CRKP growth, have been selected as the control group as well.

**Results:** In our study, it was determined that glycopeptide and steroid use, absence of tracheostomy inhibited the development of CRKP as mechanical ventilation, tracheostomy, urinary catheter presence, central venous catheterization, nasogastric tube placement, advanced age, Acute Renal Insufficiency (ARI), Total Parenteral Nutrition (TPN), carbapenem, glycopeptide, piperacillin tazobactam use was being detected as risk factor in terms of CRKP.

**Conclusion:** As a result, to remove risk factors in order to minimalize CRKP infection with rational use of antibiotics for preventing infections developing with CRKP should be aimed.
Contact isolations in South India: Guidelines vs practice

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Background: Infection control guidelines recommend implementation of standard and transmission-based precautions to control multidrug resistant organisms (MDROs) within the hospitals. However, gaps between evidence-based guidelines and practice of contact precautions in the developing world were not assessed. We report the practices of contact precautions in South Indian hospitals.

Methods & Materials: A survey and nonparticipant observation study was carried out during October 2015, using a questionnaire on contact isolations such as availability of policy on contact isolation, isolating in a single room/cohorting in ward, displaying isolation sign on the entrance of the room, availability of gowns, gloves, and hand rubs outside the room and monitoring the adherence of policy by health care workers entering the room.

Results: During study period contact isolation practices of 20 institutions were observed. Policy on contact isolation was there in 50% of the hospitals, other results are shown in table-1 below.

Table-1 Contact Isolation practices in South Indian Hospitals

<table>
<thead>
<tr>
<th>Contact isolation Policy</th>
<th>No of hospitals following/ Total No of hospitals surveyed</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Policy on contact isolation</td>
<td>10/20</td>
<td>50%</td>
</tr>
<tr>
<td>Isolation in single room</td>
<td>4/20</td>
<td>20%</td>
</tr>
<tr>
<td>Sign board at the entrance</td>
<td>6/20</td>
<td>30%</td>
</tr>
<tr>
<td>Availability of gowns, gloves, hand rubs outside the room</td>
<td>5/20</td>
<td>25%</td>
</tr>
<tr>
<td>Monitoring adherence</td>
<td>2/20</td>
<td>10%</td>
</tr>
</tbody>
</table>

Conclusion: Despite fifty percent availability of contact isolation policy, there is big gap existing in implementing the policy in South Indian hospitals. Our findings suggest that there is a need for regular auditing the practices of contact isolation policy is urgently required.
Consistent use of LLINs among household members of Kersa, Eastern Ethiopia

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Background: Though several studies have been conducted on insecticide-treated nets (ITNs) and long-lasting insecticide nets (LLINs) use in sub-Saharan Africa, the actual consistent use of mosquito nets is hardly studied. Most studies on mosquito net use also have been cross-sectional surveys. The aim of this study was to measure changes of the number of LLIN consistent user individuals and its predictors among household members of Kersa.

Methods & Materials: A longitudinal study was conducted among the household members in Kersa Demographic Surveillance and Health Research Center (KDS-HRC) from November 15 to December 30, 2010. A cohort of 1030 LLINs owned households, with their household members, were randomly selected and involved in the study. The data were collected in four waves every other week via interviews and observations. A Generalized Estimating Equation (GEE) was used for data analysis.

Results: Consistent use of LLIN declined towards the end of the malaria season. Early in the season 2236 (41.6%) individuals were consistent users and at the end of the season it declined to 10.2%. The presence of LLINs on hanged position (Adjusted IRR = 3.41, SE = 0.181, P < 0.0001), availability of an adequate number of LLINs (Adjusted IRR = 1.25, SE = 0.052, P < 0.0001), and the presence of children under five years age (Adjusted IRR = 1.24, SE = 0.078, P < 0.0001) were more likely to use LLINs consistently than their counterparts.

Conclusion: Residents in malaria endemic areas tend to be less protected at the end of malaria transmission season. Individuals tend to use bed net if it is kept in a ready to use position in the household.
Multi resistant VIM-positive Pseudomonas aeruginosa in the health care setting - Lessons learned to combat transmission

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Background: Multi drug resistant (MDR) Pseudomonas aeruginosa are increasingly seen in the hospital. Especially vulnerable patients are involved. In our 1320-beds university hospital, Rotterdam, the Netherlands, we experienced an outbreak of MDR –VIM positive P. aeruginosa.

Methods & Materials: The outbreak was investigated by identifying environmental sources, transmission routes and by implementing preventive measures. P. aeruginosa can be found in high humidity places (e.g. water taps, sinks, respiratory therapy equipment). Therefore, source environment was extended to these reservoirs or sources. Furthermore, we performed a systematic review to further elucidate sources and transmission routes.

Results: This outbreak was atypical in the number of patients affected (n= > 150) and the period of time (5-6 years). Many interventions were consecutively applied. Increase of compliance of general prevention measures was not successful. However, the ongoing transmission could be explained by persistent sources, the sinks. Measures to prevent transmission were adapted after this finding; separation of clean and dirty procedures and materials in the neighborhood of the sink, which led to a decrease in transmission. However, these measures depend highly on the compliance to keep away from this contaminated place. Disinfection of the sink and syphon was not successful on the long term.

Cultures of hands health care workers have been performed, but they all were tested negative. Environmental cultures were negative except sinks. Furthermore, device related transmission was detected and outbreak management was aimed at contacts of the device instead of contacts of patient. after the device was removed, the transmission stopped.

The systematic review and meta-analyses showed that carbapenem use and medical devices are the leading risk factors for carbapenem-resistant Pseudomonas aeruginosa. This highlights the importance of antibiotic stewardship and reduction of device days.

Conclusion: Outbreak management of MDR P. aeruginosa was more complicated than expected. This was primarily due to newly recognized sources and difficulty in removing these reservoirs. Classical contact search by looking back and screen contact patients (epidemiological relations in time and space) did not stop transmission. Therefore, in case of P. aeruginosa one of the starting points of outbreak management should be the detected reservoirs followed by a prospective and retrospective contact search.
Antibiofilm and antimicrobial activity of bacteria from hard corals and sponges in Indonesia

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\(^1\)Atma Jaya Catholic University, Jakarta, Indonesia, \(^2\)Yonsei University, Seoul, Korea, Republic of

**Background:** Antibiotic usage is the most important treatment to overcome the problem of pathogenic bacteria. Pathogenic bacteria that can form biofilm are even more dangerous than single bacterium due to high resistance against antibiotics and host immune system. Marine ecosystem is a high potential source of antimicrobial agent produced by organisms and microorganisms associated with them, which included hard corals and sponges. Therefore, the discovery of new metabolite that show antimicrobial activity as well as inhibit biofilm formation is required as alternative approach to fight infection of pathogens.

**Methods & Materials:** In this study, we screened hard corals and sponge associated bacteria with antimicrobial activity and antibiofilm activity for both inhibition and destruction activity. We used several pathogen bacteria to be tested for antimicrobial activity including Staphylococcus aureus ATCC 29213, Streptococcus pneumoniae ATCC 49619, Shigella flexneri, Vibrio cholera, Pseudomonas aeruginosa ATCC 27853, and ETEC using agar well diffusion method. While for antibiofilm activity certain isolates were analyzed against some pathogenic bacteria including Staphylococcus haemolyticus, Streptococcus pneumonia ATCC 49619, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853, Vibrio cholera C43, Enterotoxigenic Escherichia coli, Enteropathogenic Escherichia coli. Several isolates were further identified using PCR amplification of 16S rRNA gene sequencing. Characterization of the antibiofilm compound also done to classified the compound as polysaccharide, nucleic acid or protein.

**Results:** Twenty six bacteria were isolated from hard corals and sponges, and twenty of them (77%) showed antimicrobial activity against S. flexneri, S. pneumonia, P. aeruginosa, and Vibrio cholera. We also assayed the susceptibility of all the isolates against several antibiotics. It performed that 30.77% isolates were resistant to Erythromycin (10µg), 23.07% were resistant to Trimethoprim (5µg), 7.69% were resistant to Kanamycin (30µg), and 3.84% were resistant to Ciprofloxacin (5µg) and Gentamycin (10µg). Six out of fourteen marine isolates showed potential antibiofilm activity and were further sequenced to identify the isolates as well as compound characterization. One isolate showed stable results for the inhibition and destruction assay and were further characterized to identify its bioactive compounds.

**Conclusion:** Marine bacteria are potential source of antimicrobial and antibiofilm resources and this activity were promising as potential candidate for many industrial application.
Infection prevention and control - Bridging the knowledge gap among Kenyan health care workers
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Background: National policies and guidelines for infection prevention and control (IPC) have been in existence in Kenya since 2010. In addition, a national Strategic plan for IPC was developed and launched in 2014 and is currently being implemented. However, there are currently no surveillance programs in place for health care–associated infections (HAIs) or formal training course in infection prevention and control for health workers in Kenya.

Methods & Materials: This study was designed to assess the knowledge and practice of infection control among Kenyan health care workers (HCWs) in selected public health facilities. Twenty one (21) health care workers were conveniently sampled from three public health facilities. They comprised nurses (9), pharmacists (3), clinical officers (4) and laboratory technicians (5). The study comprised a fifty question pre-test prior to the administration of the basic training in Infection prevention and Control followed by a post-test after pilot testing the modules to assess knowledge transfer. None of the selected HCWs had not undergone any comprehensive training in infection prevention and control.

Results: The pre-test average score was 41%.

<table>
<thead>
<tr>
<th>IPC Parameter</th>
<th>% Knew</th>
<th>% Did not know</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application of standard precautions</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Cohorting</td>
<td></td>
<td>53</td>
</tr>
<tr>
<td>Most Commonly occurring HAI</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td>Difference between colonization and infection</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Right order of processing Instruments</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Time required to achieve High level disinfection</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Spectrum of activity of disinfectants</td>
<td></td>
<td>79</td>
</tr>
<tr>
<td>Purpose of Handwashing</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>Proper disposal of injections</td>
<td></td>
<td>79</td>
</tr>
<tr>
<td>Waste segregation</td>
<td></td>
<td>68</td>
</tr>
</tbody>
</table>

Conclusion: Although several efforts have been made to strengthen IPC programs, major gaps still exist in IPC knowledge, skills and attitudes. These gaps can be attributed to deficiencies in previous training administered which focused on specific areas of IPC like injection safety and medical waste management instead of a comprehensive approach to IPC training. Responses to questions showed specific gaps in prevention of hospital acquired infections and antimicrobial stewardship, instrument processing and sterilization procedures. The findings from this pilot training provide valuable baseline data for future interventions in providing training on infection prevention and control for health workers. An integrated yet comprehensive IPC training, is necessary to reach a wider cross section of HCWs to address the above gaps.
Active fractions from Zanthoxylum acanthopodium fruit modulate inflammatory biomarkers in lipopolysaccharide-induced macrophages in vitro
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Background: The fruit of Zanthoxyllum acanthopodium has been traditionally used as a traditional medicine in Indonesia. Our previous study demonstrated that Z. acanthopodium fruit extract exerted anti-inflammatory effect for prevention and treatment of inflammatory-related diseases.

Methods & Materials: In this study, we isolated active fractions from Z. acanthopodium fruits, i.e. polysaccharide, protein, polyphenol, and essential oil fractions, and tested their efficacies on modulating the expression of several inflammatory biomarkers, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, and matrix metalloproteinase (MMP)-9, at protein and gene levels in lipopolysaccharide (LPS)-induced macrophages by conducting ELISA and Real Time-PCR assays.

Results: Most fractions at lower dose (10-25 µg/mL) showed a significant inhibition (~50%) on TNF-α, IL-6, and MMP-9 levels in LPS-induced macrophages treated with LPS. At gene level, essential oil and polyphenol fractions (1-5 µg/mL) strongly reduced the mRNA expression of TNF-α, IL-6, iNOS, COX-2, and MMP-9 in cell system.

Conclusion: These results suggest that selected active fractions derived from the fruits of Z. acanthopodium may be considered for anti-inflammatory candidates in LPS-induced macrophage system.
Evaluation of Ondo State acute flaccid paralysis surveillance system (2009-2013)

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Background: Acute flaccid paralysis (AFP) is a clinical syndrome with infectious and non-infectious causes. AFP surveillance is the gold standard for detection of poliomyelitis cases and surveillance during polio outbreaks. Ondo state AFP surveillance system was evaluated to describe the key attributes and the process of operation, to determine the efficiency and effectiveness of the surveillance system and to make appropriate recommendations for improving the surveillance system.

Methods & Materials: The evaluation was conducted using the Center for Disease Control and Prevention (CDC) Updated Guidelines for Evaluating Public Health Surveillance System, 2001”. The evaluation included interview of stakeholders, review of relevant document and resource materials and the analysis of 2009 – 2013 data from the Ondo state AFP surveillance system.

Results: The AFP surveillance system in Ondo State is simple, flexible and sensitive (annualized NPAFP rate of 3.8/100,000 children less than 15 years in 2009, 4.0/100,000 in 2010, 4.3/100,000 in 2011, 4.1/100,000 in 2012 and 6.4/100,000 in 2013). The system is representative, stable and generates high quality data (97%, 98%, 97%, 98% and 99% from 2009-2013). Partner agencies provided 90 – 95% of the system’s operating resources. The system is acceptable to stakeholders but has a low positive predictive value (1.2% cumulatively from 2009 to 2013). The state has however not recorded any case of WPV since 2010.

Conclusion: Findings from the evaluation revealed that the AFP surveillance system in the state is meeting its set objectives of detecting Wild Polio Virus (WPV) and identifying areas with continued risk for WPV transmission. The data quality was good but the resources to maintain the system are inadequate at the state and the LGA levels. The state and local government authorities’ ownership of the initiative need to be improved.
Molecular characterization of group A rotaviral diarrhea complicated by enteropathogenic E.coli- Study of dual infections in children from Kashmir-Himalaya
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Background: Rotavirus genotypes G₁₂ & G₉ with P₆ specificity were detected among children with acute gastroenteritis in Kashmir-India. Sequence analysis of the VP7 gene indicated a high level of aminoacid homology (98-99%) to other G₁₂ strains reported worldwide, suggesting introduction of novel rotavirus strain serotype into the community. The study documents the first detection of G₁₂ strain from Kashmir-India. G & P types detected were G₁, G₂, G₉ & G₁₂ and P₄, P₆ & P₈ respectively. Escherichia coli strains that produce AE lesions and carry the eaeA gene but not stx genes are designated as EPEC. 20 cases of EPEC were reported and 15 cases were of dual nature wherein Group A rotavirus and EPEC were detected simultaneously. Dual infections complicated nature of diarrhea and treatment.

Methods & Materials: Samples collected from Kashmir Children Hospital, Srinagar. Detection by RNA-PAGE, reverse transcription, amplification of the VP4 & VP7 genes and sequencing of VP7 gene. Isolation of bacterial DNA, amplification for detection of eaeA genes.

Results: (45%) Rotavirus was prevalent in the age group of < 5 years. The phylogenetic analysis of VP7 gene sequence of G₁₂ strains shows high degree of amino acid conservation with a South African strain and other globally reported strains. The G₁₂ strains were detected during summer season. 6 cases of G₁₂P₆ were all children between 6 months to 10 months of age. (10%) cases were positive for EPEC and 7.5% were of dual nature. Subtyping of the eaeA gene showed the presence of eaeAα₁, eaeAβ, eaeAη and eaeAξ subtypes. Infants (≤ 8 months) under 2 years of age are susceptible to rotavirus infection in this study. Majority of the positive cases were suffering from loose motion with watery stools, persistent vomiting, dehydration and fever. No case of bloody diarrhea was documented.

Severe acute respiratory infections associated with influenza and non-influenza viruses - Yemen, 2011-2014

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Ministry of Health, Sana'a, Yemen

Background: In 2010, influenza surveillance started in Yemen after H1N1 influenza outbreak. Ministry of Health (MoH) established two sentinel sites for Severe Acute Respiratory Infection (SARI) at the two main public hospitals in Sana’a and Aden with the support of NAMRU3, where SARI samples tested for influenza and non-influenza viruses by the Real-Time-PCR assay. The aim is to describe the SARI severity as indicated by admission to intensive care unit (ICU) and fatality as well as associated influenza and non-influenza viruses among hospitalized patients of the two sentinel sites in order to provide recommendations for improving influenza surveillance.

Methods & Materials: Data of hospitalized patients of the two sites who meet WHO SARI case definition during 2011 to 2014 was obtained from MoH. Data was cleaned and analyzed using SPSS program where P value < 0.05 was the cut point for significance.

Results: 1,665 met SARI cases definition during 2011 to 2014, of which 64% from Aden, two thirds were below the age of two years, 48% were males, 24% has chronic diseases and 33% was admitted to the ICU. Overall fatality rate was 10% which significantly higher among patients from Aden than Sana’a (14% vs. 3%, P < 0.001). 1299 (78%) samples were tested where influenza viruses were confirmed in 67 (5%); of which 41 (61%) was type A and 27 (39%) was type B. Non-influenza viruses were detected in 39% (509) of samples including 246 (48%) Respiratory Syncytial Virus and 99 (19%) was Adenovirus. The influenza viruses was significantly higher in Sana’a than Aden (63% vs 37% P value < 0.01) while the Non-influenza virus was significantly higher in Aden than Sana’a (54% vs. 46%, P value < 0.01). The case fatality rate among non-influenza was 11% compared to 6% among influenza cases but the difference was not statistically significant.

Conclusion: Our findings showed that most SARI cases was of non-influenza type with high mortality that necessitate prompt diagnosis and treatment of suspected cases. Expanding SARI surveillance to include more public and private hospitals in different governorates is recommended to give more comprehensive picture. Further studies to better understand the geographical differences are needed.
M health technology for surveillance of infectious diseases: Challenges and learning for scale up and replication

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Background: Smart Phone based infectious disease surveillance has the potential to provide real-time, high quality, validated data for early response and investigation. However, little evidence is available on practical use of m health technology and challenges in infectious disease surveillance in lower and middle income countries. The objective of this paper is to examine the practical challenges in using m health technology in surveillance of infectious diseases and share the learning for replication and scale up of m health in surveillance of infectious diseases.

Methods & Materials: An electronic search was conducted for research articles on mobile phone based infectious diseases surveillance and the articles published only in peer reviewed journals after year 2008 were considered for including in this paper. A total of 11 research papers were selected based on this criteria and thematic analysis was done to understand challenges and learning.

Results: There were challenges in diseases surveillance using m health technologies. These were mainly technology related, financial, political and social, ethical and cultural. Technological issues were mainly - touch screen interface, application errors, and change in settings of the device. A training program in local language and handholding support in the field was found effective. Financial issues were related to procurement of smart phones, annual maintenance, repair, lost and damaged phones, software cost including application development and maintenance. Locally available hardware and open source software options should be explored. Political issues were including continued resource support and interests. Ensuring their participation from the design phase of the project has been useful for aligning the project with their priorities. Ethical, societal and cultural issues were related to data security, use of data, resistance to change and operating on mobile devices and scepticism in the community about the new technology. Building data security system, setting forth purpose of data collection and involving community in the process was useful.

Conclusion: The disease surveillance using m health technology possesses several challenges including technology related, financial, political and social. For scale up and replication, training and handholding, local technology providers, open source software and involvement of multiple stakeholders from the design phase of the project should be considered.
Animal-cell phone based surveillance and notification of infectious diseases in remote settings: A case study of plague in Uganda

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**Background:** This study aims to test effectiveness of animal-cellphone based disease surveillance and reporting model for Yersinia pestis, a primarily flea-borne bacteria that causes animal and human plague. In Uganda, Y. pestis is believed to be maintained in the wild rodents by Arvicanthis niloticus and Crocidura spp. These natural reservoirs do not develop clinical plague, unlike the domestic rat "Rattus rattus", which die in large numbers when infected. Upon deaths, rodent fleas go on rampage for alternative source of blood meals, putting humans at risk of Y. pestis infection.

**Methods & Materials:** This study utilizes Rattus rattus die offs “Rat Fall” to monitor transmission of Y. pestis in the rodent populations. Village Health Teams (VHTs) from 85 utmost risk villages were trained and equipped to safely collect and report carcasses. When village members report rodent die offs, the frontline VHTs collect and deliver to the carcass the plague laboratory. A toll free land line was installed in the laboratory for effective communication. Following a positive carcass, ecological investigation, health education and Indoor Residual Spraying (IRS) are undertaken in the case village. The study also tracks time lags between major events, right from time the rodent carcass was reported, to time when IRS or any other appropriate response is undertaken by the health authorities.

**Results:** Since inception in July 2013, 15 out of 435 rodent carcasses from 10 different villages; have tested positive for Y. pestis. The average time elapsed from VHT reporting rodent die off to VHT receiving laboratory results ranged from 1- 3 days, while days elapsed to IRS ranged from less than 5 to more than 30. Rattus rattus constituted over 76% of all reported carcasses, the others were Arvicanthis niloticus (14.5%), Crocidura Spp. (1.8%), Mastomys Spp. (1.8%), Mouse (1.4%), Zelotomys hildegardeae (0.9%), Lophuomys silkapusi (0.5%), and unidentified (3.4%). Human cases reduced drastically from 153 in 2008 to a low of 6 in 2014, and only 1 in 2015.

**Conclusion:** Partial results from this study suggest that animal-based disease surveillance and reporting models can be effective in reducing human plague in remote settings like the West Nile Uganda where the disease is endemic.
Molecular characterization of human enteric adenovirus circulating among children below five years of age in Kolkata, India

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¹National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, India, ²National Institute of Cholera and Enteric Diseases, Kolkata, India

**Background:** Human enteric adenovirus belonging to subgenus F(HAdV) are one of the most common pathogens responsible for acute infantile gastroenteritis worldwide. Subgenus F adenoviruses comprises of two distinct serotypes, viz., AdV-40 and AdV-41. In this study we aimed at detecting and serotyping human enteric adenovirus subgenus F from fecal specimens based on one to three hypervariable region of the hexon gene and partial shaft region of fiber gene respectively.

**Background on Adenovirus Infection**

**Methods & Materials:** In a hospital based surveillance study, 3085 stool specimens from children below five years of age with mild to severe diarrhea were collected over a period of two years (January 2013-December 2014). Initial screening of adenovirus was done by Enzyme Immunoassay (EIA) from the fecal suspension. Further, viral genome (DNA) was extracted from the positive fecal specimens followed by amplification of hexon and fiber genes by Polymerase Chain Reaction (PCR) using specific primers. Nucleotide sequencing of purified PCR amplicons were carried out and enteric adenovirus-F serotypes 40 and 41 were determined using BLAST search. Phylogenetic dendrograms of hexon and fiber gene were constructed and genetic distances were analysed from the previously reported adenovirus-F 40 and 41 strains.

**Materials and methods used in this study**

**Results:** A total of 56 (7.7%) and 131 (14.9%) samples out of 3085 were positive for adenovirus-F(40/41) from hospitalized cases and outpatients respectively. No discrete seasonal variation of infection was observed and age distribution revealed a greater frequency in children between 6-24 months. Incidence of HAdV-F-40 infection was found to be prevalent over HAdV-41. Phylogenetic analysis of hexon genes from AdV-41 strains revealed co-circulation of both genome type cluster-1(GTC1) and GTC2 in Kolkata, reflecting accumulation of amino acid mutations in the HVR of the hexon gene. A recombination event was evident as hexon gene of five HAdV-41 strains belonged to GTC1, whereas fiber gene clustered with GTC2 strains. Sequence analysis of fiber genes of HAdV-41 strains revealed 15 amino acid deletion from 15th repeat motif of the shaft region which has been evolutionarily conserved among Kolkata strains.

**Results of the study**

**Conclusion:** Our findings revealed the prevalence of HAdV-F serotype 40 over 41 and co-circulation of both GTCs of HAdV-41 strains among children below five years of age in Kolkata.
Is the South African notifiable diseases surveillance system effective in preventing outbreaks? Perceptions of key stakeholders

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Background: The 2014 Ebola crisis in West Africa has highlighted the importance of an effective notifiable disease surveillance system (NDSS) at country level. This study was conducted to determine the perceptions of key stakeholders on the NDSS attributes of usefulness, timeliness, simplicity, flexibility and acceptability in South Africa.

Methods & Materials: During 2015, a nationally representative cross-sectional survey of communicable diseases coordinators and surveillance officers at national, provincial and district health levels, as well as members of the National Surveillance Forum, the South African Malaria Elimination Committee and the National and Provincial Outbreak Response Teams was conducted. Individuals with less than one year experience of the NDSS were excluded from the study. Participants completed a self-administered questionnaire after giving informed consent. In addition to demographic information, the questionnaire elicited information on participants’ perceptions of the NDSS attributes. The survey was analyzed using STATA® version 14.

Results: The majority of survey participants (n=114) were from the National Outbreak Response Team, with a median of 10 years’ experience with the NDSS. On the criterion of acceptability, which measures the willingness of providers to participate in the system, a mean score of 47% was obtained. Similar low mean scores were obtained on timeliness (55%), which measures the promptness of taking appropriate action; on flexibility (55%), which measures the adaptability of the system; usefulness (61%) and simplicity, on which a mean score of 66% was obtained.

In the multiple logistic regression analysis, factors associated with positive perceptions on the effectiveness of the NDSS were participation in Provincial Outbreak Response Teams (Coefficient=10.41, p-value 0.005), participation in disease detection (Coefficient = -15.24, p-value 0.020), and participation in disease control and response (Coefficient = -16.41, p-value 0.011).

Conclusion: The overall low percentage scores on the system attributes indicate that stakeholders do not regard the South African NDSS as acceptable, flexible, simple, timely and useful. The study findings should inform the revitalisation of the NDSS in South Africa and enhancing the participation of NDSS stakeholders.
Increasing tuberculosis yield from investigation of contacts of smear positive TB cases through engagement of civil society organizations: Active TB case finding in Mombasa, Kenya

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Background: Tuberculosis (TB) still remains a public health challenge post 2015 in developing countries. Kenya notified 89,211 TB cases in 2014. This excludes about 20% of the TB cases that are missed annually by the health system (World Health Organization). Mombasa County notified 4,726 TB cases resulting in a TB case notification rate of 469 per 100,000 populations in 2014. This is far above the national average of 208/100,000. Kenya has adapted ENGAGE TB strategy and embraced Civil Society Organizations (CSOs) for implementation of community TB activities. Through CSOs, Community Health Volunteers (CHVs) are engaged in contact investigation and tracking of treatment interrupters.

Working through Amref Health Africa the principal recipient for Global Fund Grant, Christian Health Association of Kenya (CHAK) implemented activities through public health facilities. Key objective was to strengthen community systems for effective and efficient TB control interventions and reduce delays in TB diagnosis.

Methods & Materials: From August 2014 to March 2015, 126 trained CHVs implemented intensified TB case finding in Mombasa County. Households of smear positive TB clients were visited, health education provided, TB patients encouraged to adhere to medication and their contacts screened for TB. Presumptive TB cases were referred to health facilities for further investigations. CHVs collected and transported sputum for those with difficulties reaching health facilities. Documentation and reporting was done using Ministry of Health tools and reported every month. CHVs were provided with monthly stipend and bicycles to reduce mobility challenges.

Results: From 725 smear positive TB patients, 1,396 household contacts were screened. 39.2% (547) were symptomatic and referred of which 20.7% (113) were diagnosed with TB. From 13,360 general community members screened for TB, 55.3% (7,394) were symptomatic and referred of which 8.6% (636) were diagnosed with TB. Notified TB cases referred by CHVs rose from 4% to 28% during implementation of active case finding.

Conclusion: CSOs and CHVs remain key in community-based TB activities. TB yield from investigation of index smear positive TB cases is still higher than for the general community. This need to be scaled up in areas with high TB burden and expanded to work place contacts.
Bacterial and fungal infections among hospitalized patients with respiratory infections

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**Background:** The present study aimed to determine prevalence of bacterial and fungal respiratory infections as well as their clinical condition, type of microorganisms, and type of medications among patients who were hospitalized due to respiratory problems in two great referral hospitals in Iran.

**Methods & Materials:** This cross-sectional study was performed on 139 patients with median age of 67 years because of various respiratory problems who were hospitalized in Rasoul-e-Akram (n = 85) and Firouzgar (n = 54) hospitals in Tehran between 2013 and 2014. For assessing type of microorganisms including bacterial and fungal organisms, sampling was carried out with aspiration or biopsy. The primary diagnosis of microorganisms was based on gram staining and then the specimens were cultured on specific media.

**Results:** Regarding underlying disorders, 36% of subjects had the history of cerebra-vascular diseases or malignancies leading the patients to become bedridden. The final diagnosis was pneumonia in 39.6%, sepsis in 22.3%, and pneumonia plus other underlying disorders such as cerebra-vascular diseases in only 1.4%. Bacterial specimens were found in 79.1%, fungal specimens in 19.4%, both types of microorganisms in residual 1.5%. The most common microorganism found in media cultures was *Acinetobacter spp.* (28.8%), followed by *Candida albicans* (15.8%), and *klebsiella pneumoniae* (13.7%). With respect to medication, 81.3% were treated with antibiotics, 17.3% received antifungal drugs, and 1.4% received both types of drugs. The most frequent administered drugs included Azithromycin (14.5%), Colistin (13.8%), and Meropenem-Ciprofloxacin (12.3%). At follow-up, 41% of patients had second positive culture, and 27.3% died, however 22.3% were cured successfully and discharged. Moreover, 9.4% had long-term hospitalization. Fungal positive cultures was more frequent in the patients admitted to the ward of respiratory disease compared to those who were admitted to ICU wards (25.8% versus 17.6%), while positive culture for bacteria was more prevalent in the patients hospitalized in ICU wards than those who hospitalized in the ward of respiratory disease (80.6% versus 74.2%).

**Conclusion:** A notable number of patients with various types of respiratory problems suffer bacterial and fungal infections resistant to antimicrobial treatments leading prolonged hospitalization as well as high mortality and morbidity.
Mapping the awareness levels of mothers about the danger signs of acute respiratory infections in children of the Southern States of India, its relation with treatment seeking behaviour

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**Background:** Southeast Asia stands first in number for ARI (Acute Respiratory Infections) incidence. SEAR countries together with sub-Saharan African countries account for more than 80% of all ARI incidences. In India, more than 4 lakh deaths every year are due to pneumonia accounting for 13%-16% of all deaths in the pediatric hospital admissions. Identification of severe respiratory infections from non severe forms necessitates that mothers are aware of danger signs. India is a federal union of states comprising twenty-nine states and seven union territories with 676 Districts. Identifying the districts with low levels of awareness can help in prioritizing the high risk districts. An attempt is made to analyze the available data in the field of ARI to map the high risk districts in terms of Awareness among the Mothers and see its relation to Treatment seeking behaviour in Southern States of India.

**Objectives:** To Map the High risk districts with low awareness levels among mothers about the danger signs of ARI To study the correlation between Awareness of danger signs and Treatment seeking behaviour.

**Methods & Materials:** Secondary data published in the District Level Household and Facility Survey -4 is analyzed. Chloropleth Mapping of High risk districts was done. Spearman’s rho ($\rho$) is used to measure the linear correlation between variables a) Awareness of danger signs among Mothers b) Treatment seeking behaviour of Mothers and c) Utilization of Government Health Services.

**Results:** Awareness about danger signs of ARI was better in Kerala followed by Telangana and Andhra Pradesh. Mothers in Tamilnadu seek government facilities more often than those of other states for ARI management though it has most number of high risk districts. There is a Positive correlation between Awareness levels among mothers about danger signs of ARI in children and treatment seeking behaviour ($\rho=0.254; p=0.008$). The Utilization of Government Health Services is negatively correlated with the awareness levels ($\rho=-0.344; p=0.001$).

**Conclusion:** Early diagnosis and treatment is the corner stone for controlling Under-5 mortality attributable to ARI. Kerala sets an example for the rest of the southern states by its higher awareness levels and better treatment seeking behaviour following an episode of ARI.
Etiology of diarrheal disease in children from 0 to 14 years old admitted in Hospital Geral Mavalane, Mozambique

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Background: Diarrhea is one of the most important causes of infant mortality mainly in developing countries. In Mozambique, it is estimated that diarrhea is responsible for approximately 12% of deaths, however there are few published studies on the disease etiology in the country. The current study aimed to identify the main etiologic agents involved in diarrhea onset in infant patients admitted in a health facility in Maputo City.

Methods & Materials: We analysed stool samples collected from children aged from 0 to 14 years old, between May 2014 and May 2015 within the National Surveillance of Acute Diarrhea. The detection of agents was performed using immunoenzymatic assay for virus, formol-ether concentration method and the Modified Ziehl-Neelsen staining technique for parasites and culture techniques for bacteria.

Results: Stool samples of 161 children were collected and 157 tested for virus and parasites, 101 for bacteria. The detection rate of the etiological agents was 71% (114/161). The frequencies of viral, parasitic and bacterial pathogens detection were 39.4%, 37.1% and 45.9%, respectively. Rotavirus was detected in 29% (45/157) of samples, followed by adenovirus with 12% (12/104), norovirus with 6% (5/88) and astrovirus with 2% (3/157). In bacteria, Escherichia coli was isolated in 42% (42/101), followed by Shigella spp. and Salmonella paratyphi A both with 1% (1/101); and for intestinal parasites, Entamoeba histolytica/dispar was detected in 15% (23/157), followed by Cryptosporidium spp 11% (18/157), Ascaris lumbricoides 9% (14/157), Giardia intestinalis 8% (13/157), Trichuris trichiura with 4% (7/157) and Balantidium coli with 3% (5/157). The most affected age range was between 0 to 2 years old.

Conclusion: Rotavirus and E. coli were the most detected agents during the period of analysis, however, the other detected agents had a substantial influence in diarrhoea onset. The present findings show that: (1) The dynamic of diarrhoea in children involves different etiologic agents and (2) there is a need to expand the active surveillance to other health facilities of the country, in order to support the introduction of preventive measures as well as the knowledge of the epidemiologic profile of the agents.
Clinical presentation, management and outcomes of influenza in Africa: systematic review, 2009-2014
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Background: This paper aims to review the effectiveness of diagnostic and management of influenza in Africa, specifically mortality, treatment and outcomes.

Methods & Materials: In two times, we searched the online databases PubMed™ and Scopus™ for articles and abstracts published in English and French between January 2003 and December 2014, with the following terms: (influenza OR flu) AND (clinical) AND (management OR outcomes) AND (Africa) at the first time, and online databases of internationals conferences for the abstract who do not meet the consent of the editor of scientific journals at the second time. Cross-sectional, longitudinal studies and randomized clinical trial on influenza were selected when clinical, management and outcomes were reported.

Results: Patients with influenza were more likely to present with fever as initial and main symptom, followed by shortness of breathing, cough, muscle & joint pain, sore throat, hemoptysis, dyspnea, and gastrointestinal complaints (vomiting and diarrhea), pneumonic infiltrations in the chest. For the diagnostic nasal secretions were collected in patients presenting with flu syndrome and follow-up by laboratory identification of viruses was performed by the ELISA technique using anti-A and anti-B monoclonal antibodies (immunocapture) and by isolation on MDCK cells, quantitative real-time polymerase chain reaction (qRT-PCR) assay of the upper respiratory tract is used increasingly to diagnose lower respiratory tract infections. Amongst samples analyzed for influenza; 1–45% had laboratory-confirmed influenza infections; including influenza virus A (H3N2) type, A (H1N1) type, A (H5N1) type and influenza virus B. All confirmed cases received oseltamivir in any setting. Among groups known to be at high risk of influenza-associated complications, included age<5 years, asthma, cardiac disease, pregnancy, diabetes mellitus, active pulmonary tuberculosis and chronic malnutrition. Mortality rate was 28 – 68.4%. Female sex, age>15 years, and receiving the first dose of oseltamivir>2 days after illness onset, non vaccination against the virus the circulating influenza, cardiovascular complications and ventilatory associated pneumonia were identified as mortality predicting factors.

Conclusion: The classic presentation of influenza in Africa is most often confused with malaria, low technical platforms limit the detection of virus in the samples. The progressive creation of influenza sentinel surveillance system will improve care in Africa.
Prevalence of congenital malaria in Blue Nile state, Sudan

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Background: Malaria during pregnancy is a priority area for malaria research and control as pregnant women represent a high risk group for severe malaria. Additionally, malaria during pregnancy may result in fetal exposure to malaria if parasites are transmitted across the placenta and could result in congenital malaria which is defined as the existence of asexual forms of malaria parasites in the peripheral blood of an infant within 7 days of life, irrespective of clinical symptom. Recently, congenital malaria is increasingly reported among babies born to mothers from endemic areas. The aim of this study was to determine the prevalence of congenital malaria in newborn babies delivered at the maternity awards in Blue Nile State, Sudan.

Methods & Materials: Ethical approval was obtained from the ethical review committee of the Directorate of Research, Federal Ministry of Health, Khartoum, Sudan. The prevalence was studied in ElDamazin Hospital, ElRuseres Hospital and in a private clinic at ElDamazin city during Sep. 2112 – Sep. 2013. Information on mother’s demographic data was recorded in a pre-designed questionnaire. Following written informed consent, capillary blood samples were collected from babies’ heel prick within 24 hours of delivery to make thick smear for Giemsa stain, and dried spot on filter papers for nested PCR.

Results: A total of 301 mother–neonates pairs were enrolled in the study. The mean weight of all neonates was 2.72 kg and the babies with congenital malaria weighed less than 2.5 kg. The Plasmodium falciparum was the only species identified in all the babies. The prevalence for congenital malaria was 2.0% using microscopy with low parasite densities in the 6 infected babies (≤50 /µl). Moreover, nested PCR identified 41 positive samples (13.6%). Mothers of neonates with congenital malaria had at least one episode of clinical malaria during pregnancy. Furthermore, all infected infants were born from primigravid young mothers. Three positive neonates were found in each of the 2 hospitals and no positive case in the private clinic.

Conclusion: Materno-fetal transmission of P falciparum is existence in Blue Nile state that may lead to intrauterine growth retardation, low birth weight and malaria illness mortality.
Identification of etiologic agents in meningitis cases by multiplex real-time polymerase chain reaction and culture
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Background: The etiological study of meningitis has traditionally been performed using bacterial cultures and more recently using polymerase chain reaction. The aim of this study was to determine the etiological agents that cause meningitis using both traditional culture techniques and molecular diagnostic methods to improve the laboratory diagnostic assessment of the patients.

Methods & Materials: The data of cerebrospinal fluid (CSF) polymerase chain reaction (PCR) and culture results carried out on patients with clinical signs and symptoms of meningitis were examined between June 2013 and October 2015 in Selcuk University Medical Faculty Hospital. CSFs were cultured in blood culture BACTEC bottles (Becton Dickinson, USA) or/and conventional media and the bacteriae were identified by VITEK 2 system (bioMerieux, France). Total nucleic acid extraction (DNA/RNA) of CSFs was performed using the EZ1 Virus Mini Kit v2.0, Qiagen, Germany) by EZ1 Advanced XL (Qiagen Inc., Valencia, CA). Multiplex real-time PCR was performed on a Qiagen Rotor gene Q thermocycler (Qiagen, Germany) using a FTD viral meningitis and FTD bacterial meningitis kits (Fast-track Diagnostics, Luxembourg).

Results: A total of 274 cases were enrolled, and of these, 96 were children and 178 were adult patients. The etiological agents identified by molecular methods were in 38/274 (13.86%) cases and the organisms were herpes simplex virus type 1 seven cases, enterovirus eight cases, human parechoeavirus one case, Neisseriae meningitidis three cases and Streptococcus pneumoniae 19 cases respectively. By culture N. meningitidis was isolated from one case, S. pneumoniae from three, Staphylococcus epidermidis from two, methicillin-resistant Staphylococcus aureus from one, Enterococcus faecalis from one and Klebsiella pneumoniae from one case. All N. meningitidis S. pneumoniae strains isolated by culture were also detected by molecular methods.

Conclusion: Our study indicates that S. pneumoniae is the main etiologic agent for meningitis. Molecular methods are effective diagnostic tools for infectious diseases, but culture has another property having the opportunity to do antibiotic susceptibility tests. Some bacteriae also doesn’t included in the list of commercial molecular tests for meningitis. The utility of molecular diagnostics for pathogen identification combined with conventional culture methods will improve health outcomes of meningitis cases.
A Laboratory Information Management System (LIMS) for animal health: Experiences of the Istituto Zooprofilattico Sperimentale of Sicily (Italy)
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Background: Livestock diseases are a zoo-economic and public health problem, particularly in case of zoonoses such as brucellosis. Their eradication are an objective of the Veterinary Services. In Italy, this objective was pursued by specific National Recovery and Eradication Plans, providing for control and surveillance measures. This work is carried out by the National Health Service involving activities, figures and structures heterogeneous using several operational information systems. In the past, this complexity has generated confusion in data collection with partial, missing or duplicated information, often inconsistent among different systems. This made it difficult the government, control and reporting activities.

Methods & Materials: Sicily is the region with the highest prevalence of brucellosis in Italy. Brucellosis management in Sicily is currently the most stringent example of cooperation and interoperability among national, regional and local systems. The health data flow starts from Local-Veterinary-Services that use the Animal-Health-System (SANAN), interacting with the National-Livestock-Data-Base, to retrieve all information related to farms and animal master data. Samples, identified by SANAN number, arrive at Istituto Zooprofilattico Sperimentale (IZS) of Sicily where are subject to diagnostic tests. In 2014, standardization of laboratory processes and sample tracking has been increased in IZS Sicily using the LIMS "SILAB -SICILIA" developed by IZS Abruzzo&Molise.

Results: Only typing in SILAB-SICILIA the SANAN number, the loading of all information characterizing the sample is automatically activated. When the results of analysis are entered in SILAB-SICILIA, they are copied in SANAN. At the same time, data are also available to authorized operators by STUD (Diagnostic-Telematic-System) Web application. Monthly, data extracted from SILAB-SICILIA feed the National-Brucellosis-Information-System. Twice a year the data entered in SANAN are submitted to European Commission (2008/940/EC, 2003/886/EC) and feed the annual collection of Zoonoses Information System (SINZOO) which updates EFSA (European-Food-Safety-Authority) database as required by 2003/99/CE directive.

Conclusion: The tight integration among information systems has increased the quality of the data collected in each single database enabling cross-checks and allowing comprehensive reporting. It also enables to satisfy the information debts towards international organizations, provides decision-making tools for the management and governance of the National Health Service and facilitates the planning of activities, their periodic verification and risk analysis.
Trends of acute watery diarrhea in Lao People’s Democratic Republic, 2009-2013

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Background: Diarrheal disease is the second leading cause of death in children under five and account for 1 in 9 child deaths worldwide. In Lao PDR, it is estimated that 12% of the under-five mortality are due to diarrhea with acute watery diarrhea (AWD) introduced as one the national notifiable disease in 2004. Both worldwide and in Lao PDR, rotavirus is the leading cause of acute diarrhea in children under five. We aimed to describe the epidemiology of AWD from 2009 to 2013 in Lao PDR.

Methods & Materials: This was a retrospective study examining data from the national indicator-based surveillance system where health facilities send weekly reports to the national center for laboratory and epidemiology (NCLE). We collected both aggregate data (N=117,277) from LAOEWARN (Lao Early Warning And Responses Network) and case-base data (N=67,750) from line-listing from 2009 to 2013 and performed descriptive analysis using Epi info 7, Excel and ArcView GIS. We also examined the laboratory findings from 231 stool samples tested using rotavirus rapid test in 2013 from 8 diarrhea sentinel sites based in Vientiane capital.

Results: The incidence of AWD increased from 2009 to 2012 and leveled off from 2012 to 2013. We saw a seasonal trend for AWD which peaked during the dry seasons. Bolikhamxay and Sekong are the provinces with the highest reported cases of AWD. The most affected age group was children under five who were 7-9 times more likely to have AWD than the rest of the population (P<0.001); in under-fives, males are more likely to be brought to healthcare facilities for AWD than females (P<0.001). Finally, in children under-five, we detected rotavirus in 48% of the stool specimen tested at the sentinel sites.

Conclusion: The increased AWD incidence may reflect a true increase in the burden of AWD in the country or an improvement in the sensitivity of the system given LAOEWARN was only introduced in 2008. We recommend integrating hygiene and sanitation health education into nursery school and primary school and exploring risk factors for AWD during dry seasons in order to plan control and prevention strategies.
Mantle: A free and multilingual software for one health biosurveillance & research

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**Background:** The One Health approach suggests that humans, animals, and the environment are closely tied together. Human interaction with wildlife and the environment contributes to increased risk for human, plant, and animal infectious disease outbreaks. Despite the movement towards One Health, the software currently available to manage, analyze, and communicate the vast amount of One Health data is grossly inadequate. One Health data are continually growing in size and complexity, and new technologies must be developed to address the magnitude of the problem. Open access and open source software are needed to address these complex One Health problems, and to improve data accessibility, interoperability, and information communication.

**Methods & Materials:** Mantle will handle tabular data, and other widely used spatial data formats. It will visualize and explore data in useful ways, and allow data to be downloaded as the originally uploaded file or in a customizable format for use in analytical software. Mantle will store metadata—information about a dataset and its contents—using development standards for linked data (e.g., JSON-LD and WCSV, part of the overarching Resource Description Framework). Tapping into the emerging semantic web enables richer interactions with datasets, streamlining many common data tasks.

**Results:** Policymakers and decision makers will be able to view real-time visualizations of Mantle data feeds in dashboards. Researchers will be able to upload datasets representing the output of models built in other analytical software, which can be shared with policymakers, who can also view and interact with the output of custom-built modeling modules to view timely and meaningful summaries of public health data feeds. Potential use cases for the general public include browsing day-to-day textual and syndromic surveillance information, viewing the predictions of a one-time study, and monitoring the latest calculated epidemic curve in an outbreak or ongoing epidemic.

**Conclusion:** Mantle will facilitate crosscutting collaborations between disciplines and institutions. Users will be able to create, manage, and join organizations and groups. Groups of users will access and collaborate on collections of datasets, grouped manually or by specified properties. For instance, users interested in Ranavirus can view and contribute to the Global Ranavirus Reporting System, a collaborative effort by scientists worldwide to aggregate observed cases of Ranavirus across species and locations (a Mantle prototype).
Evaluation of ebola virus disease surveillance system in Tonkolili District, Sierra Leone – 2015
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**Background:** Ebola Virus Disease (EVD) remains a major public health challenge in West Africa with Sierra Leone recording more than 50% of all confirmed cases. Enhanced surveillance was commenced in December, 2014. We therefore, evaluated the surveillance system to determine the systems attributes and gaps requiring strengthening.

**Methods & Materials:** Surveillance data for Tonkolili District EVD was reviewed and descriptive analysis was performed using Microsoft Excel. Key informant interviews with the program stakeholders were done. CDC updated guidelines for evaluating public health surveillance was used. Attributes determined include Simplicity, sensitivity, Positive Predictive value.

**Results:** The system provided information and data on disease trends and outbreak report/rumours. Case definitions were well understood by participants, with willingness to continue by all stakeholders. Standardized data collection tools were in place and data communication was clear with feedback to surveillance units at all levels. The EVD surveillance was not operated within the Integrated Disease Surveillance and Response framework (IDSR). Information technology are updated frequently on suspected cases sent to the laboratory. Data completeness was about 91%, consistency exist but data quality was poor (incompletely filled data and missing data exist). Timeliness of sample getting to the laboratory either same or the following day occurred in 174(84.9%). Sensitivity of the surveillance system was 88.5%. Predictive value positive was 25.8%. The stability was questionable since the government of Sierra Leone were not fully in charge of the system.

**Conclusion:** The system could not fully meet its objectives. There is a need to channel efforts towards integrating EVD surveillance into the IDSR, improve on data completeness and timeliness. The District Health Management Team needs to take ownership of the surveillance system for sustainability.
Integration from patient registration to WHO reporting in Azerbaijan and Georgia
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Background: Multiple electronic systems are implemented in countries to strengthen disease surveillance, but they often struggle integration to achieve data completeness, quality, timeliness, transparency and reliability throughout the reporting chain [2]. Azerbaijan and Georgia provide an example of successful implementation of integrated disease surveillance solution providing transparent data reporting within country and with international entities.

Methods & Materials: Azerbaijan and Georgia implemented open source Electronic Integrated Disease Surveillance System (EIDSS) as a national surveillance tool for public health and veterinary services at more than 350 locations. EIDSS strengthens national disease surveillance providing a secure way to collect and share data in near-real time, analyze and integrate information on humans, animals and natural vectors diseases. EIDSS was integrated with the Health Management Information System in Georgia and Tuberculosis Surveillance System in Azerbaijan, which track patient visits and specific diseases at the primary healthcare locations. EIDSS was also integrated with the WHO’s Centralized Information System for Infectious Diseases in Azerbaijan and Georgia [1]. This electronic bridge since 2014 supports measles and rubella cases direct reporting from EIDSS to WHO.

EIDSS Human and veterinary surveillance networks in Azerbaijan and Georgia

Results: The vertically integrated systems overcome technical barriers through transparent notifications sharing from the primary healthcare to WHO through national disease surveillance. Data completeness and quality is improvement through system monitoring, collected data reviews and automated data entry quality control. Data accessibility is improved with reliable electronic storage and nationwide system availability on desktop, web and Android. EIDSS-WHO electronic reporting completely substituted the original manual submission process. EIDSS-FAO reporting component is under development at the moment.

Conclusion: The vertical integration of disease surveillance with EIDSS tool improves completeness, quality, timeliness, transparency and reliability (as official government tool) of reporting in Georgia and Azerbaijan and reinforces accurate international reporting including IHR. It may also consider establishing horizontal ties to support regional cooperation and data exchange.

References
Hospital based sentinel surveillance of bacterial meningitis in India
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Background: Pentavalent vaccine (a combination vaccine which protects against five killer diseases-diphtheria, pertussis, tetanus, hepatitis B and Haemophilus influenzae type B) is being introduced in various states of India as a part of Universal Immunization Programme. In this context it is critical to establish sentinel sites across India for surveillance of H.influenzae type B meningitis and use this opportunity also for surveillance of pneumococcal and meningococcal meningitis. Keeping this in mind a hospital-based sentinel surveillance network was established at 11 places across the country and this study reports the results of first three years of this surveillance program

Methods & Materials: This study was conducted in six states of India through 11 hospital based sentinel sites. Children aged one to 59 months who met with standard case definitions were included in the study and their sterile biological specimens were processed for laboratory identification of bacterial pathogens. Further characterizations of bacterial pathogens were in study reference laboratory. Reference laboratory reconfirmed bacterial identify and further characterized the isolates to identify serotypes and antimicrobial susceptibility profile.

Results: A total of 10202 cases were suspected for bacterial meningitis in study population. Laboratory confirmed incidence rates for S. pneumoniae, H. influenza and N. meningitidis in this study is found to be around 3.80%, 1.26% and 0.24% respectively. S. pneumoniae appears to be the predominant pathogen among laboratory identified bacterial pathogens with 71.7%, followed with H.influenzae (23.8%) and N.meningitidis (4.4%). For S.pneumoniae, 15% of positive cases had serotype data, showing serotypes 19F, 6B, 14, 6A and 14 were the predominant serotypes in India.

Conclusion: Invasive bacterial disease appears to be a major problem in Indian children with S.pneumoniae dominating and H.influenzae decreasing in the pathogens list. If pneumococcal conjugate vaccine-13 incorporated into Universal Immunization Programme, it can provide protection against 79% of serotypes responsible for Invasive pneumococcal diseases in India. However continued surveillance representing all parts of the country is necessary to understand the complete picture of Invasive bacterial diseases in India.
Evaluation of passive pharmacovigilance surveillance system in Tanzania – a review of secondary data

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**Background:** Tanzania Food and Drugs Authority (TFDA) routinely collects adverse drug reactions (ADRs) data to detect, prevent ADRs, protect public health, and reduce avoidable costs to the health care system through passive pharmacovigilance surveillance. Under-reporting is a challenge and limited epidemiological evaluations of the system have been conducted. This study evaluated the system to determine performance of the system in meeting its objectives and its attributes and provide recommendations.

**Methods & Materials:** CDC guidance (MMWR 2001) for evaluation of public health surveillance system was used. Review of secondary data for the period 2006 – 2013, and key informant interviews were conducted.

**Results:** Between 2006 and 2013, 630 ADRs cases were reported, 108 (1.4%) cases reported in 2013 compared to 8000 expected cases annually. All interviewed health care providers (HCPs), know ADRs case definitions, three trained on ADRs surveillance, two send reports to TFDA. Among 36 sampled reported forms, 27 (75%) were incompletely filled, 7 (19.4%) correctly filled, 9 (25%) received at TFDA timely. From January – December 2013, 108 cases were located in the database while 80 cases found in reported forms. Among seven visited health facilities, five had only yellow forms and two reported ADR cases to TFDA. Data management and analysis is done at TFDA headquarters.

**Conclusion:** The system met some of its objectives: it is flexible, stable and representative but not simple, acceptable, and has poor data quality and timeliness. HCPs and public awareness on ADRs reporting should be increased. The system should be integrated into government health administrative levels, including follow-ups. Supportive supervision and data transmission mechanisms should be improved.
Device-associated infection rates with microbiological profile and antibiogram pattern from an adult medical-surgical ICU of a tertiary care hospital

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**Background:** Intensive care unit (ICU) is considered the epicenter for health care associated infections (HAIs) due to the underlying disease severity with comorbidities, increased use of invasive interventions and wide spectrum antibiotics. Ubiquitous medical devices, continuing to be essential in permitting lifesaving treatment among critically ill patients, unfortunately are a major cause of HAIs especially in the ICUs.

**Methods & Materials:** A prospective surveillance was implemented in a 15 bedded adult medical surgical ICU of a 2500 bedded tertiary care hospital from March to August 2015. Central line-associated bloodstream infection (CLABSI), ventilator-associated pneumonia (VAP) and catheter-associated urinary tract infection (CAUTI) were defined and the DA-HAI rates were calculated using the Centers of Disease Control National Nosocomial Infections Surveillance System and National Healthcare Safety Network guidelines.

**Results:** During the study period 564 patients were admitted for 2192 days. Fifty four episodes of DAI were documented, 55.55 % belonging to age group 31-60 years with male to female ratio of 1.57. Average length of stay was 8-14 days in 37% of the 54 DA-HAI cases among whom 68.52% had a fatal outcome. DA-HAI rates for VAP, CLABSI and CAUTI were 16.74, 10.33 and 7.31 respectively with the overall DANI (device associated nosocomial infection) rate of 24.64 DAI per 1000 ICU days. The device utilization ratio was maximum for urinary catheter (0.99) followed by 0.68 for ventilator and 0.57 for central line. The overall ALOS (average length of survival) was 6.62 days whereas the crude overall case-fatality was 68.52 % for patients who acquired a DA-HAI and 38.24 % for those without a DA-HAI, yielding an overall crude excess mortality of 30.28 %. Overall 40.74% of DA-HAIs were caused by Klebsiella species followed next by Acinetobacter species in 25.93%. Cephalosporins were the most resistant (80-100%) against gram negative organisms while imipenem (79.16%) and vancomycin (75%) showed maximum sensitivity to gram negative and positive organisms respectively. No colistin or linezolid resistant gram negative or gram positive isolated was reported.

**Conclusion:** Surveillance of DA (device associated) HAIs allows a valid estimation of the effectiveness of quality improvement activities or any new infection control measure adopted.
Bacterial and fungal infections in liver transplant recipients
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Background: According to the latest WHO data, Liver disease related deaths in India reached 2.44% of total deaths. The most effective treatment for patients with end-stage liver disease is to undergo liver transplantation. Infectious complications are major causes of morbidity and mortality after liver transplantation. They might cause surgical site infections, bacteraemia, pneumonia, catheter-related infections and urinary tract infections.

Methods & Materials: This study was carried out from January 2014 to January 2015 at Institute of Liver and Biliary Sciences to estimate the morbidity and mortality associated with bacterial and fungal infections in post LDLT patients, to find the bacteriological spectrum of infections, organ system involved and to find the association of timelines post surgery with the infections. Data was collected and recorded from the date of surgery up to 6 months post surgery. Clinically significant growth post surgery was recorded and accounted.

Results: A total of 64 patients formed the study group. Of these 53(82.81%) patients were males and 11(17.1%) were females. The overall mortality was 14(21%). Post transplant bacterial infections were seen in 31(48.4%) of patients. A total of 103 episodes of infections were recorded. 6 episodes of respiratory tract infection, 6 intra abdominal, 9 episodes of bacteraemia and 1 UTI were recorded in the first week of post operative period. 11 episodes of respiratory tract infections, 8 Intra abdominal and 5 episodes of UTI and 1 bacteraemia were recorded during 1 week-1 month post transplant. 16 episodes of respiratory tract infection, 22 intra abdominal and 13 episodes of bacteraemia and 5 UTI were recorded in the next 5 months post transplant. Klebsiella species was responsible for 43(41.74%), E.coli was responsible for 20(19.41%), Pseudomonas 10(9.7%), Enterococcus 7(6.7%), Acinetobacter 11(10.67%), Stenotrophomonas 5(4.8%). Candida spp was responsible for 7(6.7%) of the infections.

Conclusion: Infectious complications remain important preventable causes of morbidity and mortality among liver recipients. The vast majority of infections that occur immediate post transplant are often related to surgical procedure, medical devices or prolonged hospitalisation. It is essential to have in place an effective approach to prevention, based on predicted infection risk, local antimicrobial resistance and surveillance of specific risk factors.
Molecular identification of beta lactamase producing gram negative bacteria in water samples collected from River Yamuna in Agra Region

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Background: River Yamuna is recoded to have microbial pollution including drug resistance pathogens. Pathogens like extended spectrum-β-lactamase (ESBL) is derived from β- lactamases and has the ability to hydrolyse the beta lactam ring of cephalosporins and other drugs. Extended spectrum-β-lactamase producing Klebsiella pneumoniae and Escherichia coli are arising as multi-national threat that could become multi-drug resistant infections. The presence of reference genes blaCTX-M, blaSHV and blaTEM coding for the β-lactamases enzymes are important marker to identify ESBL producing Klebsiella pneumoniae and Escherichia coli. This study investigated the presence of ESBL antibiotic resistance genes in Gram Negative bacteria isolated from water of river Yamuna.

Methods & Materials: DNA was isolated from sixty four water samples collected from river Yamuna (different region and time) in Agra and amplified in thermal cycler using specific primers for blaCTX-M, blaSHV, and blaTEM gene. PCR positive samples were identified phenotypically for esbls in Bauer & Kirby 1966 disc diffusion methods utilizing recommendation of Clinical Laboratory Standards Institute (CLSI) and BSAC (British Society for Antimicrobial Chemotherapy). Species of ESBLs were identified by Biochemical test and microscopic tests.

Results: Out of 64 Yamuna water samples, 4.69% (3/64), were ESBL positive. One, 1.56% (1/64), of these was positive for two genes (SHV and TEM) while other two, 3.15% (2/64), was CTXM positive (figure 1). Microbiological and biochemical test has been given in table 1-3.

Table 1: Biochemical and microscopic characteristics of ESBL positive bacteria

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Agar Media</th>
<th>Biochemical test</th>
<th>Microscopic results</th>
<th>Identification</th>
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<tr>
<td></td>
<td></td>
<td>Meth. Red</td>
<td>Vog. Pros.</td>
<td>Cit.</td>
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<tr>
<td>-ve control</td>
<td>MacConkey / Nutrient</td>
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<td>NA</td>
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<tr>
<td>2</td>
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<td>3</td>
<td>Nutrient</td>
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</tbody>
</table>

*Note: Ind= Indole; MR= Methyl Red; VP = Voges Proskauer; cit= citrate; NA=not applicable .

Figure 1: Gel showing (A) 966 bp fragment of bla TEM, (B) 1007 bp fragment of bla SHV, and (C) 550 bp fragment of bla CTX-M (M = 1.5kb DNA ladder, Bangalore Genei)
Conclusion: Yamuna water is appeared to be a reservoir of ESBLs that could become endemic threat to population. However, more environmental samples (soil and water) and blood samples from Agra could be screened to know linkage between environmental sources and clinical cases.
Detection of the emerging rotavirus G12P[8] genotype at high frequency in Brazil in 2014: Successive replacement of predominant strains

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**Background:** The continuum characterization of circulating RVA genotypes is essential to understand how vaccine introduction could impact virus epidemiology. In the present study, an unexpected rapid changing pattern of RVA genotypes distribution in Brazilian population during three followed seasons is described.

**Methods & Materials:** From January/2012 to December/2014, a total of 3441 fecal specimens were collected from collaborating centers across Southern, Southeastern and Midwest Brazil, and likely to be representative of Brazilian population. All specimens were screened for RVA using ELISA, and genotyped by RT-PCR. Differences in proportions were tested using Chi Squares. A \( p \)-value of less than 0.05 was considered statistically significant.

**Results:** RVA was detected in 19.7% (677/3441). G3P[8] remained prevalent in 2012 (37.6%, 69/185) and 2013 (40.1%, 74/186) (\( \chi^2 = 0.107, p = 0.743 \)), but declined markedly in 2014 (3.5%, 10/281) (\( \chi^2 = 71.770, p = 0.000 \)). G12P[8] was second highest strain in 2012 (22.7%, 42/185), decrease rapidly in 2013 (2.7%, 5/186) (\( \chi^2 = 26.224, p = 0.000 \)) and re-emerged as the predominant genotype in 2014 (86.6%, 243/281) (\( \chi^2 = 118.299, p = 0.000 \)). From July/2014, G12P[8] was the single genotype detected in all regions studied.

**Conclusion:** The present study raised the hypothesis of a possible G12 outbreak being in progress. Nationally, the Hospital-based Information System surveillance data confirmed the long term decline in gastroenteritis hospitalization observed in Brazil after RVA vaccine introduction. Nevertheless, the sharp increase in diarrhea hospitalization prevalence from 2013 to 2014 observed in Southern and Southeastern regions is consistent with what appears to be an outbreak of G12P[8]. Furthermore, in 2014, the FIFA World Cup was held in Brazil, and the introduction a novel RVA strain was a real threat, given large numbers of visitors from areas with ongoing G12P[8] genotype transmission. Moreover, this event occurred right before the beginning of the RVA seasonality in the country. Worldwide, the emergence of genotype G12P[8] as an epidemiologically important strain could raise new concerns for RVA vaccine development. However, despite the possible emergence of new strains, vaccination has been shown to reduce the disease incidence of RVA infection and remain below pre-vaccination levels. Continued surveillance is needed to verify the effectiveness of the Rotarix™ vaccine in Brazil together with potential emergence of unusual genotypes.
Hospital-based surveillance of enterovirus 71 in HCM City, Vietnam, 2011-2014

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Background: Human enteroviruses are classified into four species (A, B, C and D) and include over 100 serotypes. Since 1997, Enterovirus 71(EV71) usually causes self-limited infections with non-specific symptoms and manifests hand-food-month disease (HFMD) or herpangina in children. EV71 have caused severe life-threatening outbreaks in young children in Asian. EV71 circulation among Vietnamese children was first documented in 2005.

Methods & Materials: In 2011, there is a big outbreak of EV71 in Vietnam with more than 5,000 inpatients in Children Hospital No.1 (CH1), and 32 fetal cases. National Health Research Institutes cooperates with CH1 to establish hospital-based surveillance of enterovirus in HCM City in 2011. Children <10 years of age who develop HFMD and were admitted to HCM CH1 were collected throat swabs and sera. Throat swabs were used for virus isolation and Sera were used to measure neutralizing antibody against EV71 in Taiwan NHRI.

Results: Enterovirus isolate rate with HFMD-related inpatients including 38.9% (21/54) in 2011, 19.3% (79/409) in 2012, 42.0% (173/412) in 2013 and 12% (12/100) on June, 2014. Among them, EV71 positive rate from 2011 to June, 2014 were 29.6%, 6.8%, 16.0% and 5%, respectively. The age-specific seropositive rates increase from 15.2% at <0.5 years of age to 17.2, 24.0, 29.4, 58.6, 62.3, 66.1, 77.6% at 0.5-0.9, 1-1.9, 2-2.9, 3-3.9, 4-4.9, 5-5.9 and 6-6.9 years of age, respectively.


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Background: Diarrhoeal diseases are a leading cause of morbidity and mortality in children <5 years. About 40% of diarrhoea hospitalizations in children <5 years of age is caused by rotavirus. In response to a report about an increased number of diarrhoeal cases in a public hospital in Northern Cape Province, a situational assessment was conducted to confirm the existence of an outbreak, determine the cause/s and to prevent and control future outbreaks.

Methods & Materials: We conducted a retrospective review of hospital registers and patients’ files between March – July 2015 using a standardized case investigation form. We also interviewed parents of children admitted to the ward using a structured questionnaire. Stool samples were screened using the ProSpect Rotavirus ELISA and reverse-transcription polymerase chain reaction (RT-PCR) for genotyping and real-time RT-PCR for virus detection.

Results: Between 30 March and 05 July 2015, 638 diarrhoeal cases were identified. Children <5 years accounted for 50% (n=318) and adults ≥45 years for 16% (n=103) of the cases. We found that about half of the affected people were females (326/638, 51%) and there were (278/638, 44%) males affected. Two peaks were identified at epidemiological week 16 (16 cases) and epidemiological week 24 (18 cases). Of the nine children admitted in the paediatric ward, one did not receive any dose of rotavirus vaccine. Eight had received one dose of rotavirus vaccine and five of the age-eligible children had received two doses of rotavirus vaccine. Rotavirus was detected in six of the nine stools collected with G9P[8] detected in all cases. Other enteric pathogens detected include sapovirus (n=1), norovirus (n=1) and adenovirus (n=1).

Conclusion: A seasonal increase in rotavirus is a possible explanation for the observed increase in cases. A seasonal increase in rotavirus is typically seen during the winter months (April – June). We recommend that rotavirus vaccination coverage be strengthened and diarrhoeal surveillance improved through routine data collection, analysis and monitoring.
Active veterinary and entomological surveillance to assess emerging vector-borne disease risk in the Autonomous Province of Bolzano (Italy)

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Background: The Province of Bolzano (pop. 520,000), Italian Alps, is a non-endemic area for Leishmaniasis and Arboviruses. The first record of Leishmania vector Phlebotomus perniciosus was in 2008. Continuous arrivals of infected dogs from endemic Mediterranean locations however raise the risk of zoonotic transmission. The last five years have also seen growth in other disease vectors such as Aedes albopictus mosquitoes. The new epidemiological situation is being monitored.

Methods & Materials: Several surveys were performed. In 2008, phlebotomine sandflies were collected using sticky traps, identified and geographic maps of sites produced. Leishmania serology was performed on local kennel dogs. Blood samples were tested with IFAT using L.infantum promastigotes as antigen source. The serological survey was repeated in 2009 on owned dogs with the collaboration of veterinarians at health district level. In 2014, a new entomological survey focused on sandflies and on adult mosquitoes, using CDC light, BG-Sentinel and sticky traps. Geographic distribution maps were updated. RT-PCR for Flavivirus presence were performed on mosquito RNA. In 2015, a retrospective case finding of dogs positive for canine Leishmaniasis was carried out with continued surveillance of human vector-borne disease cases as foreseen by law.

Results: In 2014, vectors were collected under unfavourable weather conditions (summer temperatures under 25°C). Tiger mosquitoes were the prevalent mosquito species (85.3%) in low urban settlements, whereas autochthonous Culex spp. were ubiquitous, frequently found in rural areas at varying altitudes (247-774 m a.s.l.). Phlebotomine sandflies showed comparable densities to 2008 (0.675-3.45 vs. 0.5-5.8 specimens/m² sticky trap) and were discontinuously distributed at sites with very specific ecologies at 300-480 m a.s.l. Canine Leishmaniasis carriers are increasing. In 2008, no infected resident dogs were recorded. First cases were reported in 2009 and by 2015 prevalence was 6.1/10,000 dogs.

Four imported Dengue and two visceral Leishmaniasis cases were recorded in humans.

Conclusion: Competent vectors of non-endemic diseases such as Leishmaniasis, Dengue or Chikungunya, and West-Nile virus are now established in the northernmost Italian Alps. Although no infected vectors were collected, the risk of infection transmission should be monitored. Insect adaptation to local ecology is evident and animal/human hosts are introducing the infections.
Unexplained neurological illness in children, Malkangiri district, Odisha, India 2014

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Background: India has reported seasonal outbreaks of acute encephalitis syndrome (AES) among children leading to substantial morbidity and mortality. From 2008-2014, 44,097 cases and 5,728 deaths were reported due to AES in India. Japanese Encephalitis (JE) virus is one of the key aetiological agent for AES outbreaks in India. Malkangiri district of Odisha reported 9 deaths in 2009 and 38 deaths in 2012 among children due to AES. The current AES outbreak in November 2014 is unusual in terms of seasonality, geographical distribution and clinical manifestations in comparison to the epidemiological features of JE. We investigated to study the etiology and epidemiological characteristics of the outbreak.

Methods & Materials: Medical records of the cases admitted in District hospital were reviewed and line-list was prepared. The family members of deceased children (n=14) were interviewed. A case was defined as illness presenting with vomiting, altered sensorium and convulsions among children < 10 years of age in Malkangiri during November 2014. Serum/CSF (Cases=4) and serum (Contacts=44) samples were processed for JE IgM ELISA and RTPCR at RMRC laboratory. Entomological survey was conducted by VCRC field station, Koraput.

Results: The median age was 3 years (Range: 1.5 – 4.6 years) with female preponderance (60 %). Overall attack rate was 4% with highest among 1-3 years age group (7%) and case fatality rate (CFR) was 93%. All cases had vomiting, altered sensorium without fever and 60 % had convulsions. Blood and CSF specimens were negative to JEV, Chandipura virus (IgM & RTPCR) and also negative for WNV and Nipah antibody. Among 116 mosquitoes from six Culex species subjected to RTPCR for detection of JE virus, all were negative. In 2012, Malkangiri had reported 38 child deaths due to AES (CFR 40%) and 10/78 serum samples were positive for JE by IgM ELISA.

Conclusion: This is an outbreak of unexplained neurological illness as we could not establish the etiology. Further entomological survey and assessment of other risk factors including test for additional pathogen should be carried out to confirm the diagnosis. Training of healthcare staff, early case detection with active surveillance, and symptomatic management will effectively control the disease outbreak.
Prevalence of Methicillin-Resistant Staphylococcus Aureus (MRSA) nasal colonization among healthy AAU undergraduates
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**Background:** The colonization of different parts of body by Staphylococcus aureus has been incriminated in many disease conditions and has become a major problem in the control of both community and hospital associated infections. A healthy carrier can therefore serve as a pool for regular and consistent release of the organism to the community. This study was therefore carried out to assess the carrier status of MRSA among healthy undergraduate students of Adekunle Ajasin University, Nigeria.

**Methods & Materials:** A well structured questionnaire which captured participant's biodata and determined their suitability for the investigation was administered on each volunteer. Nasal swab samples for the culture and isolation of S. aureus were obtained from 350 apparently healthy students spread across the five faculties of the University. Samples were cultured on Manitol Salt Agar and MacConkey agar. Confirmed S. aureus isolates were screened for methicillin resistance using oxacillin disc. Susceptibility of all isolates was done on Mueller-Hinton agar using disc diffusion method.

**Results:** The volunteers were made up of 142 males and 198 females with mean age of 19.5 ± 2.1. Ninety-eight samples (28%) were positive for S. aureus out of which 9(2.6%) were screened positive for MRSA. Other organisms isolated were Klebsiella sp, Psuedomonas sp and Coagulase—ve Staphylococci. The frequency of isolation of MRSA was higher (1.7%) among the female volunteers.

**Conclusion:** A prevalence rate of 2.6% MRSA observed in this study was high enough to generate concern, since they were all healthy carriers. Personal hygiene is therefore advocated among this studied group to curb its spread.
Seroprevalence of brucellosis in different animal species of Kailali district, Nepal

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Background: Brucellosis is a contagious disease of livestock with significant economic loss. It is also a zoonotic disease, highly infectious causing undulant fever or Malta fever. Transmission occurs between animals mainly through contact with placenta, fetus, fetal fluids and vaginal discharges from an infected animal. Brucellosis has been an occupational risk for farmers, veterinarians and employees in the meatpacking business. In human, brucellosis can cause multisystemic disease with varying spectrum of symptoms. The clinical signs may in human include intermittent or irregular fever, headache, weakness, profuse sweating, chills, weight loss and general aching.

Methods & Materials: This cross-sectional study was conducted in Kailali district of Nepal during a period from September, 2012 to January, 2013. A total of 233 animal blood samples (50 Cattle, 67 Buffalo and 116 Goat) were collected and tested for Brucella antibody by plate agglutination test (PAT). Three areas of Kailali district, namely Dhangadhi municipality, Phulbari VDC and Ramshikarjhala VDC were selected for the study considering the time and financial constraint. The serum was separated in Regional Veterinary Laboratory, Dhangadhi and the tests were done at National Zoonoses and Food Hygiene Research Centre’s Laboratory in Kathmandu, Nepal. Cold chain was maintained during the transportation of samples. The test was carried out by using agglutination method and the kit developed by Human Gesellschaft fur Biochemica und Diagnostica, Germany. Statistical analysis was done by using MS-Excel 2007 and SPSS version 19.

Results: The seroprevalence of Brucellosis was 12% (28/233). Thirty two percentage (16/50) of cattle, 13.4% (9/67) of buffaloes, and 2.6% (3/113) goats were sero positive (p<0.05). Seroprevalence was higher in females (14.6% vs. 10.6%) (P>0.05) and was higher in younger cattles and older buffalo and goats (p>0.05).

Conclusion: This study showed that brucellosis exists as a potential threat in animals of Kailali district of Nepal. This could be a potential source of infection to humans. Considering the high economic losses it can impart on livestock sector and the possible human health abnormalities. So, timely facilitation of awareness generation program and adoption of proper prevention and control strategies are recommended.
Acute encephalitis syndrome and Japanese Encephalitis, status and trends in Bihar State, India

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Background: Japanese encephalitis (JE), a vector-borne viral disease, caused by a group B arbovirus (Flavivirus) and transmitted by Culicine mosquito. Acute Encephalitis Syndrome (AES) is most widely caused by Japanese Encephalitis (JE) virus. Bihar stands third in the reporting of JE cases in India and still there is a deficiency of the data coming from locales in the state. The current study was carried out to assess the status and trends of AES and JE in Bihar state and to know the status of districts in the disease, so to come up with the recommendations for its prevention and control.

Methods & Materials: Epidemiological data on the monthly basis for AES and JE in over the past 6 years (2009-2014) was collected as reported by State Health Authorities with prior necessary permission.

Results: The total numbers of 4400 cases (733 cases/year) with an average Case Fatality Rate (CFR) of 30% in aggregate of AES for the entire study period. A total 396 JE cases reported approximately 14% CFR. The peaks are shown in start and end of the monsoon months i.e. May-June and September –October for AES and JE respectively. District such as Patna, Nalanda, Jehanabad, Nawada, Gaya, Aurangabad, Vaishali, Muzaffarpur, Sheohar, East Champaran have annual incidence rate ranging from 4.7 to 25.0 per lakh population, which falls in highest incidence reporting for AES while Patna, Jehanabad, Nawada, Gaya, Lakhisarai, Gopalganj, Siwan, East Champaran and West Champaran have annual incidence rate ranging from 0.546 to 1.78 per lakh for JE. Study shows the incidence of AES and JE in Bihar is keep increasing since 2009.

Conclusion: AES and JE is an endemic problem in Bihar state with now all district reporting cases of AES and JE. Reporting and surveillance mechanism needs to be strengthened at local level also. Confidence among the private practitioners needs to be developed for reporting of AES cases which are participating less in surveillance activities. Intense IEC activities concentrate efforts for prevention and control strategy must be operationalized at sub district level and village level to increase the participation from community so that passive surveillance increase.
Surveillance of tropical infections in medical intensive care unit

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Background: Every year different parts of India are hit by seasonal fevers in the post monsoon period. These fevers include Dengue, Malaria, Scrub Typhus, Leptospirosis, Typhoid fever and some other fevers leading to very high morbidity and mortality. The clinical picture of these diseases is so overlapping that it is almost impossible to achieve differential diagnosis of these diseases in emergency and ICU settings when the time available for intervention is highly limited. This study was conducted to generate surveillance data on tropical infections of Indian population.

Methods & Materials: This, three year long, observational, cross-sectional study captured data from medical intensive care unit of a private tertiary care hospital of northern India. A total of 51 patients were screened; and, the results are based on data from 46 patients.

Results: Leptospira (41%), dengue fever (22%), malaria (7%) and scrub typhus (30%) were the four most common tropical infections. At the time of admission, 95% patients presented with fever followed by jaundice (10%). On progression, thrombocytopenia (88%), liver dysfunction (80%), renal dysfunction (75%), secondary sepsis (68%) and shock (58%) were noted in patients. Patients were treated with antibiotics, intravenous fluids and transfusion of blood & blood products. In terms of outcomes, 60% of patients survived the infections.

Conclusion: Tropical infections may prove fatal. Such mapping provides information about the prevalence and incidence, clinical presentation, multi organ dysfunction in tropical infections. Larger studies will provide robust evidence for successful management of tropical infections.
Influenza-associated hospitalizations In Maputo City - Mozambique
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**Background:** Influenza incidence, impact and risk factors constitute the keys for definition of national health
priority actions worldwide. Influenza impact and management are not consistent in most developing countries,
particularly in Africa. Thus, in order to generate local and consistent information, Mozambique has been
implementing a sentinel influenza surveillance system since 2013. This study aimed to evaluate the influenza
impact on hospitalizations, the main characteristics of patients with influenza in hospitalized pediatric and to
identify factors associated with severe outcome in Maputo City.

**Methods & Materials:** A retro/prospective data analysis of hospital admission records/logbooks and SARI
surveillance database was conducted from January 2014 to July 2015 in three sentinel hospitals in Maputo City.
We calculated the proportion of hospitalizations associated to SARI and Influenza using the size of the inpatient
population and SARI tested for influenza, respectively. Clinical management of influenza cases were analysed.
Influenza factors associated with hospitalization were assessed by comparing 138 non-influenza SARI cases
matched by age and time of hospital admission. We analyzed the time of hospitalization and outcome of
disease.

**Results:** From the 12969 hospitalizations, 5970 (46%) were children (<14 yrs). SARI-associated hospitalization
accounted for 38% (5018) of which 53% (2695) were children. From the 1006 tested specimens, influenza virus
was detected in 5.2% (53/1006) and 4.6% (44/947) of the general and pediatric inpatients, respectively. High
influenza virus activity (38/53) accured in January and April, coincidently with the first annual peak of SARI
hospitalizations. The epidemic periods were dominated by both influenza B and influenza A (H3) in 2014 (7/13
vs 8/13) and influenza A(H3) in 2015 (19/25). From 138 cases, 46 influenza-associated SARI hospitalizations
bronchopneumonia was the most frequent outcome with 64.5%, followed by breath shortage with 17.4%. The
average length of stay was 3.72±2.5 days, These results were comparable to non-influenza and no statistical
difference was found.

**Conclusion:** These findings suggest that influenza-associated hospitalization is significant in children and
reinforce the need of SARI clinical management guidelines review, especially during epidemic periods. Severe
outcomes are similar between influenza and non-influenza associated hospitalizations. Although not exclusively
associated, influenza virus should be considered in bronchopneumonia cases.
Background: Dengue fever has become a worldwide public health concern, threatening an estimated 40% of the world’s population. However, most resource and focus are still put on malaria in Africa. Dengue statuses are poorly recognized in many African countries.

Methods & Materials: This serological survey demonstrated dengue virus (DENV) transmission with serum samples collected from 78 pregnant women in the Democratic Republic of Sao Tome and Principe (DRSTP) during 2003 to 2004.

Results: Immunofluorescence assay was performed and found 31 samples (39.74%) were positive for DENV antibodies. Indirect enzyme-linked immunosorbent assay (ELISA) showed that 53 samples (67.95%) were positive for dengue E IgG, and 38 samples (48.72%) were positive for NS1 IgG. A prevalence of 35.9% was therefore determined for dengue IgG considering samples positive by all three tests. Cross-reactions with other flaviviruses were examined by indirect ELISA against Japanese encephalitis virus, West Nile virus, and yellow fever virus. Only one sample exhibited stronger absorbance against Japanese encephalitis virus and West Nile virus. Moreover, one sample was positive for dengue IgM. These results agreed with the previous researches in neighboring countries and suggested DENV circulation.

Conclusion: The study contributes to raise public awareness of dengue and support future control strategies.
Identification of potential source of vibrio cholera- A subgroup analysis from cholera outbreak of an urban resettlement colony, North India
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Background: Cholera is a potentially fatal, faeco-orally transmitted bacterial disease which is endemic in India. For centuries, outbreaks are happening every year in different parts of the country. Different types of source were found during outbreaks. This study was during recent cholera outbreak at Chandigarh, "City Beautiful of India in August, 2015 to identify potential source of infection.

Methods & Materials: It is a cross sectional study with incorporated analytical component. Standard outbreak investigation methods were used along with a semi-structured questionnaire to identify the potential source of infection. Following an index cholera case reported from Indira Colony, an urban resettlement colony of Chandigarh by the surveillance team, active disease and water surveillance was initiated and passive surveillance was further strengthened. Apart from stool culture, drinking and sewage water were tested for microorganisms. Four subgroups were made namely cholera positive (40), negative(22), untested cases (40) and non diarrhoea controls (40) and further analysis was done.

Results: Totally, 267 and 184 diarrhoea cases were identified respectively through active house to house survey and passive surveillance. Stool culture was positive in 59 cases out of 125 tested cases. All cases were managed promptly and no death was reported. All contacts were given chemoprophylaxis and mass health education to whole area. Drinking water from 3 tube wells were found positive for H₂S test and coliforms. Drinking water was determined as a potential source after analysis of place, cleanliness, chlorination, contamination and other operational issues with tube wells. Diarrhoea was more common among men (OR-2.6) and vegetarians (OR-2.36) especially among raw vegetable eaters (7.6) irrespective of place of purchase. Egg (OR-0.39), meat (OR-0.36) and fish (OR-0.31) exposure were found significantly lower among cases. Eating from street vendors and encroachment of drinking water line by toilet was not found significant.

Conclusion: Multiple source of infection is possible. Finding the source of infection is necessary to remove the root cause and prevent the future outbreak through strengthening the surveillance system. Immediate outbreak response and action is necessary to contain the disease transmission and death. Though controversial, role of chemoprophylaxis should be tested for different field settings.
Analysis of surveillance data for Hepatitis C in China: From 2005 to 2014
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\textbf{Background:} Hepatitis C virus infection represents a significant global public health problem. The disease has been listed as one of the secondary notifiable diseases in China. Early prediction of Hepatitis C is of great importance for health planning and management. Time series analysis is an effective way to understand the behavior of the epidemic infection.

\textbf{Methods & Materials:} In this paper, we collected time series data of Hepatitis C in mainland China from 2005 to 2014. Seasonality and long-term trend were explored using decomposition methods. Hepatitis C incidence seasonality are expressed by calculating the seasonal indices. To calculate seasonal indices, the entire incidences are averaged first, and then the averaged incidence is divided by the mean incidence for each month. A linear regression model is fitted to present the long-term trend of the incidence. Besides Decomposition methods, autoregressive integrated moving average (ARIMA) was used to fit a univariate time series model of Hepatitis C incidence. The incidence from 2005-2013 was used as the training set and the incidence in 2014 was used as the testing part.

\textbf{Results:} The seasonal indices are shown in Figure 1. The Hepatitis C incidence series peaks in March and reached a lowest level in February. The linear regression model of deseasonalized series against time \(t\) is formulated as: \textit{Deseasonalized incidence as }\textit{t}\textit{= 0.303+0.011t}. ARIMA (1,0,1) \times (1,1,1) was selected as the best fitted model for modelling and forecasting. The fitting and testing, incidence of the ARIMA model for 10 years are graphed in Figure 2.

\textbf{Conclusion:} Time series models developed in the current study indicate that disease surveillance data can be utilized to understand the behavior of Hepatitis C over time. The Hepatitis C time series showed strong seasonality and increasing long-term trend. ARIMA model fitted and tested Hepatitis C incidence well. Supported by the National Science and Technology Major Project (grant number: 2012ZX10004201-006)
The analysis of infectious disease surveillance data based on fuzzy time series method
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Background: Urgent health-policy decision is usually required in the surveillance of infectious diseases. Traditional time series analysis models, such as autoregressive integrated moving average (ARIMA) model and its variants, rely on a large amount of historical data and certain model assumptions so that they may not be adequate for such situation, let alone to deal with the perception-based data usually represented by linguistic values or words. Therefore, it is quite important and necessary to build models that could account for such problems.

Methods & Materials: The fuzzy time series (FTS) method has made a wide success in finance, management, education, etc. This work described the basic idea and procedure of FTS method in a way closely relating to the epidemiological background. The incidence data of typhoid and paratyphoid fever in China from 2004 to 2014 was used as an example. Two simulation studies were also carried out to test if the FTS method could still work when the historical data were inaccurate and insufficient.

Results: The root mean square error (RMSE) was utilized as a measure of performance. The real example showed that though the fitting performance of FTS method and ARIMA model were almost the same (RMSE=0.0307 for FTS and 0.0319 for ARIMA), the FTS method outperformed the ARIMA model in forecasting with lower RMSE (RMSE=0.0243 for FTS and 0.0293 for ARIMA). Moreover, Simulation 1 displayed that even when 15% of the historical data were not accurate, the RMSEs of FTS were still lower than those of ARIMA model (fitting: 0.0314 for FTS and 0.0345 for ARIMA; forecasting: 0.0316 for FTS and 0.0347 for ARIMA). Finally, Simulation 2 showed that the ratios of insufficient-data RMSE to that of the sufficient-data were 1.44 in fitting, and 1.77 in forecasting, which suggested the robustness of FTS method.

Conclusion: FTS method could incorporate human perception into model-building. It is plausible to believe such incorporation could improve the accuracy of time series model. Therefore, it could be concluded that the FTS method may serve as a good alternative to traditional time series models under certain circumstances in disease surveillance.

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Epidemiological study of scarlet fever in Sichuan province, China

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Background: Scarlet fever is an infectious disease caused by group A Streptococcus, which is currently ranked as a second-class notifiable disease in China. The increasing of Scarlet fever incidence represents a growing concern to medical providers, policy makers and community populations. Strengthening infection surveillance and study of incidence behavior of Scarlet fever is of great importance to help devise health plans to decrease the occurrence and associated patient burden.

Methods & Materials: In this study, we collected Scarlet fever surveillance data in Sichuan province, China. Sichuan province locates in the central-western China, which covers an area of 485,000 square kilometers, and a population of 81.07 million. The average temperature of Sichuan province is 15.3°C, and the average annual rainfall is 992.1mm. It is dry and cold in winter, and hot and rainy in summer. Descriptive epidemiological analysis is conducted on the scarlet fever cases collected from the infectious disease surveillance system.

Results: The annual incidence and cases of Scarlet fever were reported in Sichuan province in 2014. The ratio between male cases and female cases was 1.4:1. The seasonal distribution of Scarlet Fever from 2012 to 2014 is shown in Figure 2. Scarlet incidence peaks from April to July. 94.63% of the scarlet fever cases were under 10 years old. The geographic distribution of Scarlet Fever Incidence in Sichuan province is shown in Figure 3. The incidence of Scarlet fever in the capital Chengdu was 5.14/100,000, which had the highest incidence of the province.

Conclusion: The incidence of scarlet fever had obvious seasonality in Sichuan province. Children are the main victims of scarlet fever. Effective preventive measures should be carried out in the kindergartens and primary schools. The study is complete and is of especial interest to epidemiologists.
Molecular diagnosis and antifungal susceptibility profiles of rare isolates of filamentous fungi among patients with cancer from South India

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Background: The spectrum of mycotic disease continues to expand well beyond the familiar entities of candidiasis and aspergillosis. Major advances in anticancer treatment have contributed to an increased frequency of severe fungal infections in patients with neoplastic diseases. Incidence due to filamentous fungi becomes life-threatening and their diagnosis and treatment can be challenging, especially in species with intrinsic resistance. Diagnosis of filamentous fungi in both the clinic and the laboratory remains difficult, leading to unsatisfactory treatment and high mortality rates.

Methods & Materials: A total of 40 clinical specimens including surgical tissues, biopsies and wound swabs were collected from Cancer patients and cultured on fungal isolation medium. The presence of fungal elements on tissue specimens were demonstrated using PAS stain by histopathological analysis. The positive fungal isolates were presumptively identified based on macroscopic and microscopic features and species were further confirmed by PCR amplification of ITS followed by sequencing the amplicons. The Antifungal susceptibility profiles of fungal isolates to Amphotericin B and Itraconazole was determined by microbroth dilution assay as per CLSI guidelines.

Results: Out of 40 specimens collected, 16 were positive for filamentous fungi. Based on phenotypic identification and ITS sequencing 9 were found to be rare isolates and were identified as Geotrichum candidum (03), Curvularia verruculosa (01), Alternaria alternata (01), Fusarium incarnatum (01), Syncephalastrum racemosum (01), Penicillium oxalicum (01) and Onychocola canadensis (01). The MIC values of Amphotericin B and Itraconazole ranged from 0.0625 to >16 µg/ml. All the isolates were found to be susceptible to Amphotericin B and Itraconazole, while G. candidum showed resistance to Itraconazole and single isolate of O. canadensis showed resistance to both the antifungals.

Conclusion: The results obtained in the study showed the occurrence of rare fungal isolates such as G. candidum, C. verruculosa, A. alternata, F. incarnatum, S. racemosum, P. oxalicum and O. canadensis in cancer patients. The Amphotericin B and Itraconazole were found to be more effective antifungals against all the species tested except few rare isolates with intrinsic resistance. However, therapeutic success in cases of fungal infections primarily depends on early diagnosis of the fungal infection, correct identification of the etiologic agent, and timely antifungal treatment.
Thirty five years scenario of cryptococcal meningitis: An analysis in pre and post HIV era

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Background: Cryptococcal meningitis remains one of the leading causes of morbidity and mortality among immunosuppressed individuals, particularly those with HIV infection. Globally, approximately one million cases occur each year resulting in more than six lakh deaths. Although incidence of cryptococcal meningoencephalitis has declined in patients who have access to anti retroviral therapy, it still remains a prominent cause of morbidity and mortality in developing world where access to ART is limited.

We have analysed the cases of cryptococcal meningitis over a period of thirty five years in pre and post HIV era.

Methods & Materials: This retrospective and prospective study was done in the department of neuromicrobiology, NIMHANS, a tertiary neurocentre of South India, from January 1979 to December 2014. Diagnosis was based on india ink preparation, antigen detection, and culture by standard laboratory methods. Serotype, mating type and genotype were determined by molecular methods in few of the isolates.

Results: In 1988, first case of HIV associated cryptococcal meningitis was diagnosed in NIMHANS. During 1996 to 2003, HIV positivity in cryptococcal meningitis was 94-96%. The incidence of cryptococcal meningitis among HIV negative patients has been on the rise reaching upto 17.25% in 2014.

A total of 1230 cases of cryptococcal meningitis were analysed, of which ten were from the pre HIV era (1986). The age group ranged from 2-76 years, and the disease was most prevalent in the 30-40 age group. Onset of the clinical symptoms ranged from 4 days to 6 months. Headache was the commonest symptom followed by high or intermittent fever and vomiting. Patients also had neck stiffness (90%), altered sensorium (65%), behavioural changes (50%), blurring of vision (30%) etc.

CSF cell count ranged from 0-400 cell/cumm with predominatly lymphocytes. India ink was positive among 90% and fungal culture was positive in all the cases.

Among C. neoformans, 4-11% belonged to biovar. gattii. Of the isolates tested, serotype ‘A’ was predominant and all were of mating type alpha. 97.5% isolates were sensitive to amphotericin B and 90.2% to fluconazole.

Conclusion: A high index of suspicion is needed for early diagnosis as many would recover with timely and adequate antifungal therapy.
Cryptococcus meningitis and the genotypes of cryptococcus neoformans prevalent in Western Maharashtra, India

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Background: Cryptococcal meningitis is an AIDS defining illness. However the disease is seen in patients without HIV infection also. Attempts have been made to develop a vaccine for the disease, so the prevalent genotypes of Cryptococcal neoformans need to be identified. In the Indian scenario there is a paucity of literature on MLST genotyping. Genotyping of C. neoformans can be performed for epidemiological purpose, to determine whether the cryptococcosis is caused by single genotype or two or more genotypes and to develop vaccine production. The present study was therefore undertaken to determine the prevalence of cryptococcal meningitis in this region, to evaluate the different diagnostic techniques available and to study the prevalence of the genotypes of C. neoformans in this region.

Methods & Materials: Cerebrospinal fluid was collected from 150 patients with clinically suspected cryptococcal meningitis from Apr 2010 to 2012. All samples were subjected to microscopy, antigen detection, culture and PCR. Twenty six of the isolates obtained by culture were further subjected to MLST genotyping method on ABI 3130 Genetic analyzer.

Results: Of the 150 suspected patients 47 were positive by culture. 43.43% of the isolates were from HIV+ve patients. Comparing the Latex agglutination and PCR techniques using culture as the gold standard, they gave a sensitivity of 91.49% and 100% respectively and a specificity of 92.92% and 87.38%. Latex agglutination and PCR detected an additional seven patients 13 patients respectively which were not detected by culture. The predominant molecular type was VNI (96.15%). Only one isolate was of VNII. The phylogenetic analysis showed three major clusters.

Conclusion: Cryptococcal meningitis a critical illness in patients on antiretroviral therapy. There could be a role of developing vaccines directed against VNI genotype of C. neoformans for the management of patients with HIV infection.
Antifungal prophylaxis with posaconazole suspension versus tablet in pediatric patients after hematopoietic stem cell transplantation

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Background: Pediatric patients after hematopoietic stem cell transplantation (HSCT) have a high risk of invasive fungal infection. Due to the excellent results from prospective studies in adults, we have been using posaconazole for antifungal prophylaxis in pediatric patients for several years now. In addition to posaconazole oral suspension, posaconazole has recently been formulated as a tablet. In this analysis safety, feasibility, initial data on efficacy and posaconazole serum concentrations of posaconazole suspension were compared to posaconazole tablet in pediatric patients after HSCT.

Methods & Materials: 52 pediatric patients with hematological cancer with a median age of 11 years (range 6 months – 21 years) that received posaconazole as antifungal prophylaxis after allogeneic HSCT were analyzed. Of the 52 patients, 31 received posaconazole suspension and 21 received posaconazole tablet up to a maximum of 200 days after HSCT. Posaconazole trough levels were analyzed on days 2, 3, 5, 7, 10, 14 and four weeks after start with posaconazole.

Results: No possible, probable or proven invasive fungal infection occurred in both groups. On every analyzed time point after start of antifungal prophylaxis the trough levels were significantly higher in the tablet group compared to the suspension group. For example: day 3 suspension group (median 133 ng/ml, mean 156±81 ng/ml, range 45-312 ng/ml) vs. tablet group (median 516 ng/ml, mean 656±385 ng/ml, range 224 - 1383 ng/ml) P<0.0001; day 7 suspension group (median 252 ng/ml, mean 390±459 ng/ml, range 54 - 2441 ng/ml) vs. tablet group (median 710 ng/ml, mean 910±528 ng/ml, range 329-2227 ng/ml) P<0.0001; day 14 suspension group (median 529 ng/ml, mean 643±493 ng/ml, range 115-2081 ng/ml) vs. tablet group (median 834 ng/ml, mean 1076±628 ng/ml, range 404-3060 ng/ml) P=0.0031; 4 weeks suspension group (median 634 ng/ml, mean 732±408 ng/ml, range 290-1664 ng/ml) vs. tablet group (median 1367 ng/ml, mean 1720±973 ng/ml, range 582-4066 ng/ml) P=0.0001.

Conclusion: Posaconazole suspension and tablet are comparably effective in preventing invasive fungal infections in pediatric patients after HSCT. Trough concentrations in the tablet group were significantly higher than in the suspension group at all analyzed points in time.
Randomized Clinical Trial on Evaluation of The Effect of Bergamot oil on Treatment of Ring Worm Infection in Calves and Cats.

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Background: Dermatophytes are one of the important fungal diseases affecting wide range of animal species including cattle, buffaloes, sheep, goats, dogs and cats. It has a severe zoonotic impact as it induce several affections in human beings. The disease is highly contagious and present all over the world. The dermatophytes include three genera, Trichophyton, Microsporum, and Epidermophyton. Dermatophytes are grouped according to their habitat as being either anthropophilic, zoophilic or geophilic as mentioned by Achterman and White 2012.

The objectives of our study are to evaluate the efficacy of local application of different concentrations of bergamot oil on the recovery of clinical cases under the field conditions.

Methods & Materials: The study was carried out on 20 calves and 20 cats suffered from clinical signs of dermatophytosis. These animals were exposed to visual examination and skin scraping of the lesions accompanied with fungal culture using Fungassay technique for detection of causative fungi. Diseased calves and cats were divided into 4 groups (5 animals /each group). Three groups were treated locally using Bergamot oil as ointment with different concentrations 1.25%, 2.5% and 5%. The fourth group was kept as control. The diseased cases were evaluated visually and by fungal culture weekly.

Results: Trichophyton verrucosum was identified as a causative agent of dermatophytosis in calves. Microsporum canis was identified as a causative agent of dermatophytosis in cats. All treated groups recovered either after 2 treatments as in 5% concentration or 3 treatments with 2.5% and 4 treatments in 1.25% with 1 week interval between each treatment. control group remains infected even after 6 weeks.

Conclusion: Bergamot oil ointments with different concentrations give good results on clinical cases of dermatophytosis either due to Microsporum or Trichophyton infection with some differences in the duration of recovery. Higher concentrations gave rapid recovery with disappearance of scales and erythema with rapid appearance of hair and return to normal skin than lower one.
Virulence determinants and antifungal susceptibility pattern of yeast flora from droppings of Gallus gallus domesticus

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Background: Poultry (Gallus gallus domesticus) meat is widely consumed globally. Knowledge on the virulence and drug resistance among yeast microbiota of G domesticus droppings is lacking. This work was dedicated towards exploring these issues.

Methods & Materials: A total of n=103 specimens of fresh bird droppings of broilers and chickens were collected from breeding site. Eighty eight yeast isolates were identified by conventional methods including morphological, physiological, biochemical and Vitek based identification. Virulence factors like biofilm formation, cell surface hydrophobicity (CSH), superoxide dismutase, protease, lipase, phospholipase, DNAse and hemolysin were studied. Antifungal sensitivity testing was performed using broth microdilution (CLSI, M27-A3/S4) for planktonic cells and XTT (2, 3Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) reduction assay for their biofilm counterpart.

Results: The isolates comprised of Candida famata (29;33%), C. ciferrii (12;13.6%), C. albicans (10;11.4%), C. catenulata (8;9.1%), C. tropicalis (6;6.8%), C. krusei (3;3.4%), C. pintolopesii (2;2.3%), and C. parapsilosis (1;1.1%), Trichosporon spp. (9;10.2%), Geotrichum candidum (4;4.5%), Cryptococcus macerans (3;3.4%) and Rhodotorula minuta (1;1%). Wide variation in the distribution of virulence factors was observed amongst different species. No major statistically significant relationship was found among the virulence factors of yeast isolates, as there were very low Pearson correlation coefficients (P value >0.05). The only observable relationship was found between the Pz values of Phospholipase and DNAse (P= 0.038) and between the Pz value of lipase and percentage of CSH (P= 0.040). Biofilm cells showed higher MICs (µg/ml) than planktonic cells against all antifungals tested: amphotericin B, 0.5 -64 vs 0.031- 16; caspofungin, 0.062- 4 vs 0.031-1; fluconazole, 8-512 vs 0.031-16; voriconazole 0.062- 16 vs 0.062- 8.

Conclusion: Detection of drug resistance and wide range of virulence factors amongst Gallus gallus domesticus intestinal yeast flora is of great concern because these flocks may be potential reservoirs for transmission of drug resistant yeasts to humans. In addition, possible horizontal transfer of virulent genes among poultry and human pathogens may pose a grave risk to human health.
Disseminated phaeohyphomycosis presenting as chromoblastomycosis in an immunocompetent host: A rare manifestation
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Background: Phaeohyphomycosis is a rare, deep fungal infection of the skin and subcutaneous tissues caused by dematiaceous fungi. Exophiala spinifera, one of the dematiaceous fungi causes local and disseminated phaeohyphomycosis. It usually occurs in persons with decreased host immunity, although few cases have been reported in apparently immunocompetent patients. We report a case of disseminated phaeohyphomycosis caused by E. spinifera in a 52-year-old woman without evidence of immunodeficiency presenting with clinical features of chromoblastomycosis.

Methods & Materials: A 52-year-old female from Tirunelvelli, Tamil Nadu with no co-morbidities presented with multiple verrucous, well-defined plaques encompassing lesions of varying sizes on her face (Fig.1), back as well as subcutaneous swelling on right hand, both legs of 5 years' duration. The lesion first manifested as non-itchy, small, erythematous papular lesion on the forehead gradually increasing in size. There was no history of apparent trauma. In last 3 months she had developed swelling in both legs associated with discharge. She was treated earlier with voriconazole with no response. There was no history of fever, cough with expectoration, loss of appetite or loss of weight. Systemic examination revealed no abnormality.

Results: Clinical diagnosis included disseminated chromoblastomycosis with chronic osteomyelitis. She underwent extensive debridement of lesions of both legs and tissue/pus sent for cultures. Biopsy revealed granulomatous infiltration composed of neutrophils and multinucleated giant cells. H&E and PAS staining showed pigmented septate hyphae proliferating in and around granulomas and budding yeast form of fungus (Fig. 2). Isolate was sent for definitive identification, susceptibility testing and molecular typing to the Center of Advance Research in Medical Mycology, PGI, Chandigarh. Incubation on Sabouraud’s glucose agar medium yielded grey-black and yeast-like colonies in which the hyphae were morphologically expanding with the formation of annelloconidia (Fig.3), indicating that the isolate was an Exophiala species. The patient was started on oral itraconazole and terbinafine. An improvement was observed 2 weeks after commencement of treatment, leading to gradual, but dramatic, resolution of the lesions.

Conclusion: This case highlights various manifestation of E. spinifera presenting clinically as chromoblastomycosis & histopathologically as phaeohyphomycosis in an immunocompetent adult with excellent response to itraconazole and possibly lower sensitivity to voriconazole.
Clinical diversity in central nervous system cryptococcosis

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Background: Though cryptococcal meningitis (CM) is recognized as a disease of the immunocompromised, studies have implicated that it also affect immunocompetent patients.

Methods & Materials: This was a cross sectional study conducted in the Department of Medicine of a tertiary teaching institution in North India. All the patients diagnosed with cryptococcal meningitis on the basis of detection of cryptococcal antigen or the presence of capsulated budding yeast cells on India ink preparation, from April 2009 to March 2015 were included in the study. Demographical profile, clinical presentation, predisposing factors, CSF characteristics, imaging abnormalities and in patient outcome were noted and analyzed.

Results: Among the 40 patients diagnosed with CM, 62.5% of them were males. Eight patients were immunocompetent, 10 had predisposing factors other than HIV and 22 had HIV infection (initial presentation in 59%). Mean age of presentation was 44.75 +/- 15.78 years. Mean duration of symptoms in all three groups varied from 3-4 weeks.

Clinical presentations included fever (16), headache (14), altered sensorium (16), seizures (5), paraparesis (4), hemiparesis (2), lateral rectus palsy (3), VII nerve palsy (2), bilateral vision loss with ptosis (1) and ataxia (1). Neck stiffness was present in 50% patients of immunocompetent group, 45.45% of HIV patients and none in the 3rd group.

Acellular CSF (37.5%) was not unusual. Mean CSF white cell count in HIV patients, in other immunocompromised patients and immunocompetent patients were 159.09 +/- 317.42, 36.88 +/- 92.43 and 32.5 +/- 62.05 /mm^3 respectively which was predominantly lymphocytic. Mean CSF protein were 136.73 +/- 139.82, 62.67 +/- 51.11 and 152.29 +/- 218.24 g/dl in these groups. Abnormalities detected on imaging included, meningeal enhancement, encephalomalacia, infarct, cerebellitis, hydrocephalus, cord hyper intensities and cervical spine intramedullary lesion.

Mortality rate in cryptococcal meningitis patients was 20%. On mortality analysis, death was mostly attributed to the primary disease.

Conclusion: Clinical presentation of CM in both immunocompetent and immunocompromised patients was similar. Though previous studies noted less inflammation in immunocompromised patients, in this series HIV patients had a better inflammatory response in terms of CSF pleocytosis compared to other groups. Since the presentation of CM is variable, all cases of meningitis should be screened for the same.
Oral candidal carriage and their antifungal susceptibility pattern in potentially malignant disorders

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Background: Significant numbers of oral squamous cell carcinoma develop from potentially malignant disorders (PMDs). Candida has been extensively studied in leukoplakia, erythroplakia, Oral submucous fibrosis and Lichen planus. Candida infection has been linked with malignant transformation ever since it was reported to cause candidal oral leukoplakia. The improvement of epithelial dysplasia following elimination of Candida spp. from infected tissue is added evidence. Eradication of Candida spp. in the oral cavity of patients with PMDs may help in preventing PMDs to malignant transformation. Hence this study was aimed to assay antifungal susceptibility test on Candida spp isolated from PMDs.

Methods & Materials: Forty four (42.30%) Candida albicans and 11 (10.57%) non albicans Candida spp (C.tropicalis, C. rugosa, C. stellatoides) were isolated from saliva of patients with clinically diagnosed oral potentially malignant disorders (n=104). The isolates were identified by phenotypic methods (CHROMagar, germ tube formation, chlamydospore formation, sugar assimilation & sugar fermentation tests /KB006 HiCandida Identification Kit) and molecular methods (ITS region amplification by PCR –RFLP). Antifungal disk diffusion susceptibility testing was performed on the isolates as per CLSI guidelines on Mueller-Hinton agar with 2% glucose and 0.5µg/ml methylene blue. C. albicans MTCC 3017 was used as control strain. The isolates were assayed for triazoles (Fluconazole and Itraconazole), imidazoles (Ketoconazole and Miconazole) and polyenes (Nystatin and Amphotericin B).

Results: C.albicans was the predominant isolates. Majority of C.albicans and non albicans Candida spp were highly susceptible to azoles, while all isolates were susceptible to polyenes. Five isolates (11.36%) of C.albicans and one non albicans Candida spp (9.09%) (C. rugosa) were resistant to triazoles. Two non albicans Candida spp and a single isolate of C.albicans were resistant to ketoconazole, while all isolates were highly susceptible to miconazole. In addition resistant mutants of C.albicans to ketoconazole, Fluconazole and Itraconazole were observed among 15 samples.

Conclusion: The present study highlights the increased prevalence of oral candidal carriage in PMDs and their resistance to azoles. Prompt diagnosis and antifungal screening may help eradication of oral Candidal carriage in preventing PMDs to malignant transformation. Azole resistance has to be further determined by genotypic method and broth dilution assay.
Detection of invasive fungal infections with broad range panfungal primers and molecular beacons in a real time quantitative polymerase chain reaction
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Background: The increasing incidence of Invasive Fungal Infection in cases of immunosuppressed patients and organ transplant recipients requires rapid methods for detection of same. Fungi are difficult to culture and this effectively delays the administration of appropriate therapy to the patient. Antigen detection assays have been promising on this score but shows variable sensitivity and specificity. Molecular detection by Real time PCR was carried out and compared with the clinical criteria for invasive fungal infections.

Methods & Materials: A total of 72 samples (Blood, BAL, CSF, Ascitic fluid) from patients suspected to be suffering from invasive fungal disease as per EORTC/MSG clinical criteria. Blood / CSF/ BAL Fluid samples were inoculated in automated culture system MycoF bottle; Flagged samples were sub-cultured onto appropriate fungal culture media for further identification. Serological antigen (Galactomannan, b-D-Glucan and Candida Mannan) detection assays were done simultaneously. Samples were also processed in parallel for DNA extraction using commercially available kits.

Real Time Quantitative amplification of Fungal DNA was carried out using published panfungal primers targeting the Inter Transcribed Spacer (ITS) regions of the 18S rDNA region of the fungal genome and molecular beacons capable of detecting medically relevant fungi.

Results: Invasive Fungal Infection was documented in 72 cases (8 proven, 29 probable and 35 possible). PCR was positive in 21 cases (6/8 in proven cases; 12/29 in probable cases; 3/35 in possible cases). PCR was negative in blood samples drawn from 72 healthy controls. Based on the positive results for proven cases of IFI sensitivity, specificity, positive predictive value and negative predictive value of PCR were 75%, 76.6%, 28.6% and 96.1% respectively.

Conclusion: Real-time panfungal PCR is a promising tool for the early diagnosis of IFI in immunosuppressed patients. It may be most useful as a screening method in high-risk patients and will help in the decision to either treat or withhold early pre-emptive antifungal therapy in patients with neutropenia.
Epidemiology and in vitro susceptibilities of candida albicans isolated from HIV patients in South India

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Background: Candidiasis is frequently seen in HIV infected patients. We studied the distribution of Candida species in HIV patients and determined the in vitro antifungal susceptibility of Candida albicans by broth microdilution method (BMD) and E-test.

Methods & Materials: Candida isolates from oral cavities of HIV patients were speciated by standard tests. Antifungal susceptibility tests were done by BMD and E-tests. Reference strain of C. albicans (ATCC 90028) was also included.

BMD was performed using RPMI 1640 buffered with MOPS, according to the CLSI method M27 A2. MIC end points were read after 24h. Drug-free and yeast-free controls were included. The E-test was performed as specified by the manufacturer (AB Biodisk, Solna, Sweden) on solid RPMI 1640 medium using a 0.5 McFarland standard cell suspension of the test organism. Strips impregnated with antifungal agents were placed on the agar surface, incubated at 35ºC for 48h and the results were read.

Results: A total of 233 Candida species were obtained from 247 HIV cases. Of the 233 Candida species, 72(30.9%) were C. albicans, and 161(69.1%) were non albicans species such as Candida krusei (27.5%), Candida tropicalis (14.6%), Candida guilliermondii (13.3%), Candida glabrata (8.6%), Candida parapsilosis (2.1%), Candida rugosa (1.7%) and Candida kefyr (1.3%).

Of the 71 C. albicans tested by BMD, 42/71(59.2%) were susceptible to fluconazole, 25/71(35.2%) were susceptible to itraconazole, 47/71(66.2%) were susceptible to voriconazole, all the strains (100%) were susceptible to amphotericin B.

Of the 71 C. albicans tested by E-test, 42/71(59.2%) were susceptible to fluconazole, 44/71(62%) were susceptible to itraconazole, 48/71(67.6%) were susceptible to voriconazole, and 70/71(98.6%) were susceptible to amphotericin B.

Conclusion: According to our findings, E-test method is easy to perform and could be an option for routine antifungal susceptibility testing. However for certain antifungal agents such as itraconazole, there was a discrepancy in the results obtained by the two methods and in such cases BMD may be preferable.
Microbiological profile of mycotic eye infections at a tertiary care institution in the Caribbean: A retrospective analysis

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Background: This study identifies the aetiological agents associated with mycotic eye disease in samples sent for fungal investigation, to the Department of Microbiology, University of the West Indies (UWI).

Methods & Materials: A retrospective analysis of mycotic eye disease was conducted using microbiology records of all eye related specimens received in the mycology section from January 1998 to December 2014. The study population was comprised mainly of hospitalized patients, those seen in the ophthalmology clinics at the University Hospital of the West Indies (UHWI), as well as those seen by private physicians. The frequency and distribution of the causative agents of fungal eye infections were ascertained.

Results: Microbiology records showed that 312 samples were received from 254 patients during the 17 years being examined, with a clinical diagnosis of fungal eye disease. A total of 52 of these samples were positive and the microbiological diagnosis was established in 39 (15.4%) of these patients (26 males, 13 females). Filamentous fungi such as Aspergillus spp n=17 (32%) and Fusarium spp n=16 (31%) were the most frequently isolated. They were followed by Penicillium spp n=5 (9%), Acremonium spp and Aureobasidium spp n=3 each (6%), Curvularia spp and Scopularopsis spp n=2 each (4%), Cladosporium spp, Papulospora and Mucor spp n=1 each (2%). No yeasts were identified. The ≥ 50 years age group had the most isolates (10/39).

Conclusion: Filamentous fungi such as Aspergillus spp and Fusarium spp are the most common causative agents of fungal eye infections in this setting. Consideration should be given to the reviewing of the first line empiric agents used for treatment of clinically diagnosed fungal eye infections locally. Empiric agents to consider should include agents demonstrating high in-vivo activity against moulds. Topical natamycin should be considered as first line for cases of fungal keratitis while voriconazole should be considered in cases of endophthalmitis. Continued surveillance is necessary to identify the introduction of other agents e.g. yeasts such as Candida spp, commonly found in other locations.
Oral candidiasis in patients with type II Diabetes: Comparision of a novel multiplex PCR and chromagar in species identification

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Background: Diabetes mellitus is a global epidemic. Oral candidiasis is being frequently recognized in diabetic patients, due to elevated glucose in their oral fluids and immune dysfunction. Oral candidiasis is associated with multiple pathogens including *C.albicans*, *C.parapsilosis*, *C.glabrata* and *C.tropicalis*. The aim of this study was to evaluate a multiplex PCR as a rapid diagnostic tool for identification of above four oral Candida pathogens.

Methods & Materials: A multiplex PCR was optimized to identify *C.albicans*, *C.parapsilosis*, *C.glabrata* and *C.tropicalis* in concentrated oral rinse samples of patients with diabetes, attending the Endocrinology clinic at Colombo South Teaching hospital, Sri Lanka. Common reverse primer, ITS4 and four species specific forward primers targeting ITS 1 and ITS2 regions of yeast genome (primer CA, CT, CP, and CGL respectively) were used. Oral rinse samples (n=100) were used to compare between multiplex PCR and phenotypic identification.

Results: Out of the 100 oral rinse samples, 77 were culture positive and of these 44 were colonized (> 600 CFU/ml). Multiple Candida species including *C.albicans*, *C.parapsilosis* and *C.tropicalis* were identified in 33 of the colonized samples. Eighty two patients were positive for Candida by multiplex PCR and of them 47 had multiple Candida species. All 44 colonized specimens were also positive by multiplex PCR. *C.albicans* was the most predominant organism (73/82) followed by *C.parapsilosis* (46/82), *C.tropicalis* (16/82) and *C.glabrata* (6/82). In specimens with multiple species, the two most common organisms were *C.albicans* and *C.parapsilosis*.

Out of the 61 Candida isolates that were germ tube negative, 15 isolates were identified as *C.albicans* by the typical green colour on CHROMagar. However two out of fifteen isolates were negative for *C.albicans* by multiplex PCR indicating that results of CHROMagar should be interpreted cautiously. Further *C.glabrata* could not be identified using phenotypic identification but was identified by multiplex PCR. Multiplex PCR yielded a sensitivity of 10 Candida cells/ml of oral rinse sample.

Conclusion: Multiplex PCR is found to be rapid, highly sensitive and specific than phenotypic identification methods in discriminating multiple Candida species directly in oral rinse specimens.
Epidemiological and etiological diagnosis of suppurative keratitis in Vadodara, Gujarat, India.

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**Background:** Corneal blindness is a major public health problem in India and infections constitute a major cause, second only to cataract. The purpose of this study was to identify the specific organisms responsible for suppurative keratitis, and to determine the risk factors predisposing to suppurative keratitis in Vadodara, Gujarat.

**Methods & Materials:** All patients with suspected suppurative corneal ulceration presenting between 1st January 2014 to 30th November 2014 to Ophthalmology department at S.S.G.Hospital, Vadodara were evaluated. The corneal scrapings were performed for cultures and smears by using standard protocols and information pertaining to risk factors was recorded.

**Results:** In the 11 months period, 66 patients with suspected corneal ulceration were evaluated. Corneal cultures were found to be positive in 33 (50%) patients. Of the 66 patients, 19 (28.70%) were diagnosed as bacterial keratitis and 14 (21.30%) as fungal keratitis. The most common fungal pathogen isolated was Aspergillus spp, representing (42%) of all positive fungal cultures, followed by Candida albicans (21%). The predominant bacterial pathogen isolated was Staphylococcus aureus representing (31.57%) of all positive bacterial cultures, followed by Pseudomonas spp (21.07%).

**Conclusion:** Suppurative keratitis is one of the leading causes of blindness in rural India and most often occurs due to superficial corneal trauma, accounting for 84.84% of culture positive cases. In patients of corneal trauma with vegetative matter which was the most common traumatic agent, 78.57% developed fungal keratitis whereas 36.84% developed bacterial keratitis (total of 64.28%). Aspergillus spp was responsible for most of the fungal infections and Staphylococcus aureus accounted for the majority of bacterial infections. It is imperative to know the local etiology of keratitis in a particular region particularly if diagnosis is going to be reliant on clinical signs for the prompt treatment of suppurative keratitis. This study helps in finding the most prevalent microorganisms in this region.
Rising prevalence of dermatophytosis in India: A matter of concern

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**Background:** Dermatophytosis (Cutaneous Mycoses) have been reported worldwide as the most common cutaneous infectious diseases among humans in clinical practice. In spite of therapeutic advances in the last decades, the prevalence of cutaneous mycoses is still increasing. The skin constitutes the main site of recognizable fungal infections in humans. We aim at evaluating the possible contributing factors in the rise of prevalence of the disease.

**Methods & Materials:** This is a multi-centric study carried out in one district of Eastern India for a period of 6 months. 83,457 patients were screened and 26348 (31%) patients were enrolled in the study. Cutaneous fungal infections were diagnosed among 16346 (62%) patients by a dermatologist. They were grouped into recurrent fungal infections (74%) and primary infection (26%). A detailed history was taken from each subject and pre-structured questionnaires were used to obtain the details like occupation, income, hygienic practices, contact history, previous treatment, and livestock contact. This data was then evaluated to assess the risk factors contributing to the rise in prevalence of Dermatophytosis.

**Results:** Hot and humid climate, poor hygienic practices, previous family history, livestock contact, prolonged work hours and immunocompromised status were the risk factor to develop the disease. Apart from the above mentioned risk factors each patient in the 1st group with recurrent fungal infection had a history of treatment failure, attributing to steroid abuse, inadequate dosage of anti-fungal, lack of counselling and loss of follow up. Relapse and recurrences were more in people visiting public places like salons, spas, gyms, and using common toilets in spite of proper treatment. All patients belonging to the 2nd group with primary infection had a definite history of contact.

**Conclusion:** Dermatophytosis compromises almost 30% of the patients attending dermatology outpatient department. From our study we concluded that patients with recurrent fungal infections act as the source of infection. Hence, to sum up poor diagnosis, improper treatment and administrative negligence contributes to the rise in prevalence of Dermatophytosis in India.
Burden of pneumocystis pneumonia in HIV-infected adults in sub-Saharan Africa: A systematic review and meta-analysis

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Background: Seroprevalence data and clinical studies in children suggest that the burden of pneumocystis pneumonia (PCP) in Africa may be underestimated. A more accurate knowledge of the epidemiology of PCP will inform interpretation of diagnostic tests, development of empiric treatment guidelines and allocation of scarce resources. We performed a systematic review to determine the prevalence and attributable mortality of PCP amongst HIV-infected adults in sub-Saharan Africa.

Methods & Materials: We searched Pubmed, Web of Science, Africa-Wide: NiPAD and CINAHL, from January 1 1995 to June 1 2015, for relevant studies that reported the prevalence, mortality or case fatality of PCP in HIV-infected adults living in sub-Saharan African countries. Prevalence data from individual studies were combined by random-effects meta-analysis according to the Mantel-Haenszel method. Data were stratified by diagnostic method and study year.

Results: We included 48 unique study populations comprising 6884 individuals from 18 countries in sub-Saharan Africa (Figure 1). The pooled prevalence of PCP among 6018 patients from all clinical settings was 15.4% (95% CI 12.9 – 18.0) (Figure 2), and was highest amongst inpatients, 22.4% (17.2 – 27.7) (Figure 3). More cases were identified by bronchoalveolar lavage, 21.0% (15.0 – 27.0), compared with expectorated, 7.7% (4.4 – 11.1), or induced sputum, 11.7% (4.9 – 18.4) (Figure 3). There was a trend of decreasing PCP prevalence amongst inpatients over time, from 28% (21 - 34) in the 1990s to 9% (8 – 10) after 2005 (Figure 3), while the proportion of inpatients on ART increased from 3.6% (1.8 – 6.6) to 24.3% (9.8 – 38.7). The case fatality rate was 18.8% (11.0 – 26.5), and PCP accounted for 6.5% (3.7 – 9.3) of study deaths. 29.5% of the 474 PCP cases in whom additional testing was performed had a confirmed co-existent opportunistic pulmonary disease; TB was most common (14.8%), followed by bacterial pneumonia (8.7%) and Kaposi sarcoma (4.1%).
Forest plots of proportion of patients with PCP by clinical setting, mortality and time period (A) and by diagnostic method (B) PM post mortem, resp respiratory BAL bronchoalveolar lavage, PCR polymerase chain reaction, Exp expectorated, Ind induced

**Conclusion:** PCP is an important opportunistic infection amongst HIV-infected adults in sub-Saharan Africa, particularly in those admitted with respiratory symptoms. Improved access to antiretroviral therapy and non-invasive diagnostics, such as PCR, are needed.
Epidemiology and treatment outcome of mucormycosis in Khuzestan, Southwest Iran

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Background: Mucormycosis is an uncommon life-threatening fungal infection. The major risk factors of this infection include uncontrolled diabetes mellitus, prolonged steroid therapy, persistent neutropenia, haematological malignancies, autoimmune disorders, trauma, burn, and surgical wounds.

Methods & Materials: This retrospective study was performed during a period of 10 years from April 2004 to March 2014. Demographic data, laboratory data, clinical features, antifungal treatment, the need for surgical debridement, and the outcome were collected and analysed.

Results: Data from 20 patients [13 males (65%), 7 females (35%)] with a biopsy-proven diagnosis of mucormycosis were collected. The mean age was 51.4 ± 9.7 years. Eighty-five percent of patients had uncontrolled diabetes mellitus. The most common site of involvement was rhinocerebral region, occurring in 6 cases (30%). All the cases received amphotericin B, but surgical debridement was performed on 10 patients (50%). The most common involved sinuses were maxillary in 9 cases (45%). The most common affected cranial nerve was the 3rd one, occurring in 13 cases (65%). The most prevalent season of mucormycosis was winter (40%). All of patients who underwent surgical debridement plus antifungal treatment survived, compared to 50% of those who received antifungal treatment alone (100% vs. 50%). Patients who underwent surgical debridement survived more than who received just medical management (P=0.033).

Conclusion: Our study showed that mucormycosis could be a serious and potentially fatal infection, especially in diabetic patients with poor control, and surgical management seems to be necessary for better outcome.
Incidence of healthcare associated infection in neurosurgical patients

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Background: Health care associated infections (HCAIs) are important public health-related issues in developing countries. There is limited data available on the incidence and burden of nosocomial infection (NI) in the neurosurgical patients of South-East Asian region.

Methods & Materials: We carried out a prospective HCAI surveillance for 3 months duration, on all neurosurgical patients admitted to a tertiary-care center of India. HCAIs were identified using the National Healthcare Safety Network definitions of CDC. The site-specific nosocomial infection rates and device utilization ratios were calculated.

Results: A total of 245 patients with 4054 patient-days were analyzed for HCAI. Fifteen NIs were identified in 15 patients. The overall NI rate was 6.12% and 3.7 per 1000 patient-days. Catheter-associated urinary tract infection (CA-UTI) was the most common (86.67%) NI followed by ventilator-associated pneumonia (VAP; 6.67%). The CA-UTI rate was 15.78 per 1000 urinary catheter-days. VAP rate was 1.69 per 1000 ventilator-days. No central line-associated blood stream infection was identified. The ratios of urinary catheter, central line, and ventilator utilization were 0.20, 0.06 and 0.15, respectively. K. pneumoniae (38.5%) was the commonest organism causing CA-UTI followed by E.coli (30.8%), Pseudomonas spp (15.4%) and P. vulgaris (7.70%). All the isolates (100%) were found to be multidrug resistant.

Conclusion: This study generates a baseline data for the record of device-associated infection, which will further help monitoring its trend and antimicrobial resistance pattern. Moreover, this study will help formulating the antibiotic policy in terms of prophylaxis and preventive measures, which may reduce the morbidity and mortality in neurosurgical patients.
Carbapenem-resistant acinetobacter species: An emerging nosocomial superbug

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\textbf{Background:} \textit{Acinetobacter} species are a heterogeneous group of organisms that have emerged as significant nosocomial pathogens affecting patients with impaired host defenses. Newborns, particularly are very prone to hospital acquired infections. Till date, \textit{A. baumannii} has been predominantly implicated in neonatal sepsis but “other species” within the genus have been reported rarely. The persistence of endemic \textit{Acinetobacter} strains in hospitals seems to be related to their widespread resistance to antimicrobial agents. Since carbapenems are last resort antibiotics, this study evaluated the dissemination of carbapenem-resistant genes in cases of neonatal septicemia.

\textbf{Methods & Materials:} \textit{Acinetobacter spp.} from blood of septicemic neonates was identified by ARDRA and susceptibility tests were carried out by VITEK 2 system. Extended-spectrum β-lactamases, AmpCs, oxacillinases and metallo-β-lactamases were detected phenotypically and genotypically and were subsequently sequenced. Pulso-typing and plasmid characterization were also carried out.

\textbf{Results:} \textit{A. baumannii} was found to be predominant genospecies (72%) followed by \textit{A. calcoaceticus}, \textit{A. Iwoffii}, 13TU, \textit{A. junni}, 15TU, \textit{A. haemolyticus} and 14TU over the 7 years period. Fifty four percent of the total isolates were meropenem-resistant. Comparative analysis of susceptibility tests revealed that carbapenem resistance (meropenem, imipenem) and aminoglycoside resistance (amikacin, gentamicin) was significantly lower in other species of \textit{Acinetobacter} against ACB (\textit{A. calcoaceticus-baumannii}) complex. The most prevalent oxacillinases were OXA-51-like (74%), OXA-23-like (50%) and OXA-58-like (15%). New Delhi metallo-β-lactamase-1 was the dominant MBL (22%). NDM-1-harbouring isolates also possessed \textit{bla}_{VIM-2}, \textit{bla}_{PER-1}, \textit{bla}_{VEB-2}, \textit{bla}_{CTX-M-15}, \textit{armA}, \textit{aac(6')Ib}, \textit{aac(6')Ib-cr} gene in various combinations. These isolates were clonally diverse (Figure 1) and harboured plasmids of different sizes.

\textbf{Cluster analysis of NDM-1 harbouring isolates}

\textbf{Conclusion:} This study documents the emergence of \textit{Acinetobacter spp.} in the nosocomial environment and also the significance of different genospecies of \textit{Acinetobacter} causing neonatal sepsis over an extended period of time. A unique assessment of the diverse genetic determinants for carbapenem resistance was noted. The emergence of NDM-1 among an already existing repertoire of oxacillinases is a challenge for clinicians and microbiologists alike. As new resistance mechanisms constantly evolve both laboratory detection systems and infection control measures need to be enhanced.
Prevalence of hospital acquired blood stream infections and its microbial pathogens in a tertiary hospital in Oman
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**Background:** This study aimed to explore the prevalence rates of Hospital Acquired Blood Stream Infections (HA-BSIs) by patients and isolates during the defined period in a tertiary care hospital, Sultan Qaboos University Hospital (SQUH), in Oman.

**Methods & Materials:** Cross-sectional study during the period from January 2013 to December 2013. Data about patients admitted at the tertiary hospital were retrospectively collected during the study period. The cumulative frequency and prevalence rates of HA-BSIs by patients and isolates were calculated. In addition, cumulative frequency of participant with single versus mixed infections and types of causative micro-organisms of HA-BSIs were obtained.

**Results:** Among the 30,764 patients analyzed, HA-BSIs occurred in 268 patients, revealing a prevalence rate of 8.7 patients per 1000 admissions. The majority of patients with HA-BSIs had monomicrobial infection. The most common isolates were *coagulase-negative staphylococcus* (21.4%), *klebsiella pneuminae* (12.3%), and *candida species* (9.4%).

**Conclusion:** This study provides a benchmark for the epidemiology of HA-BSIs in Oman. This rate is lower than rates reported by most developing countries. Further reinforcement of infection prevention and control measures are necessary to reduce these rates further.
Antibiotic prophylaxis for early ventilator associated pneumonia in patients with stroke

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Background: Ventilator-associated pneumonia is one of the most important problems in intensive care units. Different methods have been used so far to alleviate this problem. Administration of prophylactic intravenous antibiotic is one of the methods that has been used lesser. Piperacillin-tazobactam combination combines a broad-spectrum penicillin and a beta-lactamase inhibitor. The aim of this study is to prevent ventilator-associated pneumonia by the combination of piperacillin-tazobactam.

Methods & Materials: In this clinical trial study, 84 patients in intensive care unit entered the study. Patients were randomly divided into two groups. Patients in the case group received piperacillin 4 gr and tazobactam 0.5 gr in time of intubation and also 12 hours later. The incidence of pneumonia and mortality rate was recorded at the end of study.

Results: The incidence of early-onset pneumonia was 11.9% in case and 33.3% in control group (p=0.011). The incidence of late-onset pneumonia was 42.8% in the case and 50% in the control group (p=0.084), although there was no statistically significant difference between the two groups, but the incidence of late-onset pneumonia was 7.2% lower than the control group. The mean duration of hospitalization was significantly greater in control group who did not receive prophylactic antibiotics (p=0.026), while the difference in length of stay in ICU (p=0.117) and duration of ventilation (p=0.088) was not significant but the average length of time that the patients in the case group were under mechanical ventilation was 8 days lower than the control group and length of stay in the intensive care unit in the case group was 9 days less compared the control group that is economically important. Finally at the end of first week 4 patients (9.5%) in the case group and 9 patients (21.4%) in the control group died (p=0.131).

Conclusion: Using prophylactic piperacillin-tazobactam could significantly decrease early-onset pneumonia. Despite the fact that this combination could reduce the incidence of late-onset pneumonia and mortality in the intervention group compared to the control group, but this difference was not significant. It seems that this combination can be used to decrease early ventilator-associated pneumonia and reduces hospital and ICU length of stay.
Etiology, clinical course, and antimicrobial resistance of bacterial agents of ventilator-associated tracheobronchitis in surgical and medical intensive care units in Hamedan, Iran

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Background: Ventilator-associated tracheobronchitis (VAT) is an important cause of mortality and morbidity in the hospitalized patients in the ICUs. Appropriate and early onset antibiotic therapy leads to better outcome. This study was conducted to determine the frequency of bacterial agents and antimicrobial resistance, clinical course and response to treatment of VAT in the hospitalized patients in a surgical and a medical intensive care unit (ICU) of teaching hospitals in Hamedan, Iran.

Methods & Materials: In a cross-sectional study in 2014, hospitalized patients who had the criteria for the diagnosis of VAT in medical ICU of Sina Hospital and surgical ICU of Besat Hospital in Hamedan were enrolled. Tracheal samples of patients were investigated in terms of smear, culture and antibiotic sensitivity. Furthermore, demographic characteristics, underlying diseases, clinical aspects, progression to pneumonia and response to the treatment were collected by checklist. Data were analyzed by using SPSS-16.

Results: In this study, 69 patients were included, of whom, 28 patients (40/6%) were female and 41 (59/4%) were male. The incidence of VAT was 6/44%. The mean age of the patients was 55/92 ± 21/98 years. The most isolated bacteria consisted of Acinetobacter baumannii (30/4 %), Pseudomonas aeruginosa (20/3 %), and Enterobacter (13 %). In surgical ICU, Pseudomonas aeruginosa and Enterobacter spp. were the most common isolates. In medical ICU, Acinetobacter baumannii and Klebsiella pneumoniae were the most common bacteria. Over all, 63/3 % of the isolates were multidrug resistant, out of which 71 % related to the medical ICU and 29% to the surgical ICU. All the isolates of Acinetobacter baumannii and Citrobacter freundii were multidrug resistance. Also, 23 patients (33/3 %) progressed to pneumonia. The mean time of response to treatment was 4/98 ± 4.7 days, and 27/5 % of the patients were discharged after tracheostomy. Thirty-eight patients (98.6%) died in spite of antimicrobial therapy.

Conclusion: Multidrug resistant pathogens are common causes of VAT. A high proportion of VAT patients lead to pneumonia and death. Considering the difference between the kind of pathogens and antibiotic resistance in different ICUs, it is necessary to utilize the intended data of each region for defining the appropriate empirical treatment protocol.
Nosocomial infection in an intensive care unit of a tertiary hospital in Nigeria: A 4 year review

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Background: Infection is one of the major factors that determine clinical outcome among patients requiring intensive care unit (ICU) support. The attending mortality is high and depends on source of infection, organisms associated, timeliness and appropriateness of the treatment received.

Methods & Materials: Case records of patients who were admitted into our 5bedded ICU over a 4 year period were retrospectively reviewed. The average number of admission was 20 per month. A preformed questionnaire was administered and data on clinical and microbiological profile of all the patients with documented infection was obtained.

Results: Eighty four episodes of infections were identified in 76 patients. Road traffic accident 29/76(38.2%) and pulmonary embolism 12/76(15.8%) were the leading cause of admission. The most common infection was skin and soft tissue infection (SSI) 30/84(35.7%) followed by UTI 23/84(27.4) and primary bacteraemia 18/84(21.4) with the least being VAP 3/84(3.6%). Most of the cases of UTI occurred in patients with RTA and renal failure, while the highest number of primary bacteraemia was seen among patients with burns and renal failure. The most frequent isolates were S. aureus 35/84(41.7%), K. pneumonia 18/84(21.4%) and E. coli 13/84(15.5%). K. pneumonia 2/5(40%) and P. aerugenosa 1/5(20%) were the leading cause of pneumonia, while S. aureus was the commonest cause of SSI 16/30(53.3%), primary bacteremia 10/18(55.6%) and line infection 5/5(100%). There were 3 cases of VAP one each caused by K. pneumonia, P. aerugenosa and S. aureus. High rate of resistance to cloxacillin 19/35(54.3%) and co-trimoxazole 17/26(65.4%) was noted among the S. aureus isolates. All the enterobactrecae isolates that were tested against meropenem were fully susceptible, while resistance rate to ceftriaxone was high; E. coli 5/9(55.6%), K. pneumoniae 10/14 (71.4%) and proteus spp 2/4 (50%). With exception to meropenem and colistin, ciprofloxacin had a better resistance profile against P. aerugenosa and isolated enterobactriae compared to the other tested B-lactam agents.

Conclusion: The study underscores the need to improve infection control practice in our ICU. Resistance to commonly used antibiotic was high and this makes the choice of empiric antibiotic difficult, hence measures to curtail the emergence of resistance pathogens need to be adopted.
Risk Factors, outcomes and profile of central line associated bloodstream infections in a tertiary care referral PICU in South India

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**Background:** Indian data on CLABSI in PICU is scarce. Most of the reported studies are on CRBSI or they have shown combined adult, pediatric and neonatal data.

**Methods & Materials:** We conducted a retrospective study of PICU records of the 5-year study period (January 2010 –December 2014). Patients with Central Venous Catheters (CVCs) were identified. Patients with incomplete medical records or CVCs in situ for duration less than 48 hours were excluded. CLABSI was identified by the CDC definition. Demographic, disease related and catheter related factors were compared between the CLABSI and non-CLABSI groups. Outcomes studied were length of hospital, PICU stay and mortality. The profile of organisms causing CLABSI was studied.

**Results:** A total of 248 patients satisfied the inclusion criteria. 55 patients were excluded (Incomplete records= 14, Duration of CVC < 48 hours = 41). 21 CLABSI occurred during the study period. Multiple CVCs, neurological diagnoses and longer duration of CVCs were associated with increased risk of CLABSI. The mean time to CLABSI was calculated as 8 ± 3 days (5-11 days). Duration of PICU stay (p=0.0001) and duration of hospital stay (p=0.002) were significantly higher for the CLABSI group, though organ dysfunction or mortality was not higher.

Gram negative organisms (n=17) caused 81% of the CLABSI, followed by Candida species (n=3, 14.3%) and gram positive organisms (n=1, 4.7%). Pseudomonas species were the top isolates (n=6, 28.5%) followed by Enterobacteriaceae - E.coli (n= 3 ; 17.6% ) and Klebsiella (n =3; 17.6%). 3 (17.6%) isolates of Acinetobacter species were isolated. Only 11.7% (n=2) of the gram negative organisms were sensitive to cephalosporins. Carbapenem resistance was seen in 17.6% (n= 3). All Candida species were azole sensitive and the lone gram positive isolate was MRSA.

**Conclusion:** CLABSI were associated with prolonged PICU and hospital stay. Limiting the number of CVCs per patient and duration of catheterization to less than 8 days along with intense infection control measures are needed to reduce CLABSI rates in our PICU. The profile and drug resistance patterns reported here are very different from that quoted in the Western literature.
Prevalence of meticillin resistant Staphylococcus aureus and coagulase negative staphylococci bacteremia and nasal carriage in a tertiary care hospital, South India

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Background: Nosocomial infection is a major problem globally. Prevalence and antibiotic resistance of meticillin-resistant Staphylococcus aureus (MRSA) strains are reported to be increasing worldwide. MRSA and meticillin resistant coagulase negative staphylococci (MRCoNS) are the important agents causing nosocomial infections.

Methods & Materials: A retrospective study conducted for a period of three years. The study was conducted to find out the prevalence rate of MRSA and MRCoNS in nasal swabs and also from patients with bacteremia from hospital inpatients who were also subjected to nasal sampling. All isolates were identified by Clinical and Laboratory Standards Institute (CLSI) guidelines and antibiotic susceptibility pattern determined by modified Kirby Bauer disc diffusion method.

Results: A total of 7212 (inpatient and outpatient) nasal screening samples were included in the study in which 1871/7212 (25.95%) were Gram positive cocci. Of them, 467/7212 (6.48%) S.aureus, 1404/7212(19.45%) CoNS were isolated. 140/467(29.98%) S.aureus were MRSA and 473/1404 (33.69%) CoNS were MRCoNS.

Out of total 2172 tested, 371(17.08%) cases had bacteremia of which 213/371 (57.41%) had Gram positive bacteremia-49 S.aureus (23.0%) and 164(76.99%) CoNS. Of the 49 S.aureus, 26 were MRSA (53.06%), and of the 164 CoNS, 90 (54.87 %) were MRCoNS.

Out of total 2172 tested, 26 (12.21%) were MRSA bacteremia and 90 (42.25%) were MRCoNS bacteremia. Of the 371 with all bacteremia(gram positive(213)+gram negative(158)) and 213 Gram positive bacteremia, the percentage of MRSA bacteremia was 7% and 12.23% respectively.

Corresponding nasal sampling could be done in only 72 bacteremic patients, of whom 6/72(8.3%) had MRSA bacteremia and 24/72 (33.33%) had MRCoNS bacteremia.

Among the corresponding tested group, 5/72(6.95%) had nasal MRSA and 21/72(29.16%) had nasal MRCoNS. 4/6(66.66%) had MRSA and 8/24(33.33%) had MRCoNS in both blood and nose and rest of the 60(dual tested) patients had differential isolation of gram positive/gram negative/both organisms.

Conclusion: There is need for continuous monitoring of the antimicrobial susceptibility pattern of meticillin resistant staphylococcus aureus and meticillin resistant coagulase negative staphylococci in bacteremic patients and also for nasal screening and selection of evidence driven appropriate therapy, developing the antibiotic policy and for limiting the use of restricted antibiotics.
Sepsis registry in a tertiary care hospital – A 9 month observational study

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**Background:** Adult sepsis in India poses a major challenge for clinicians and hospitals given the complete lack of local and national data of its public health magnitude. Severe sepsis is often associated with high mortality and morbidity leading to increased cost of care for patients and institutions. To better respond to the clinical and financial cost of sepsis care in our tertiary care hospital we developed a sepsis registry based on the Surviving Sepsis Campaign.

**Methods & Materials:** A computer based real time sepsis registry was developed to collect data of all sepsis patients presenting to the ED. This registry included demographic information, pre-hospital care, clinical information and patient care details as per the Surviving Sepsis Campaign guidelines. This database was aligned with our hospital laboratory information system. Frequent reviews of the data collection process, quality and completeness were done to optimize the registry.

**Results:** Out of a total 301 patients, 66% (199/301) were males. 44% (131/301) were between 61-80 yrs while 34% (102/301) were between 41-60yrs of age. 40% (120/301) of the cases were transfers from nearby hospitals to our tertiary care. Further categorization revealed 46% cases were ‘sepsis’, 14% ‘septic shock’ and 40% were ‘severe sepsis’. The SOFA score on admission of the 65% of the cases were < 9 while 19% had a SOFA score of 9-10 and 16% had a score more than 11. 51% (153/301) had an average length of stay < 7 days. The primary focus of infection were pneumonia (39%), UTI (38%) and skin and soft tissue infections (16%). 22% of the cases had concomitant bacteremia. Compliance with the 3hr bundle was present in 83% of the cases. Overall mortality was 28% (86/301) with male gender and a SOFA score of > 9 significantly associated with fatality (p<0.05). Lack of compliance with 3 hr bundle and lactate levels > 2.5 mmol/L were also associated with mortality (p<0.05). 71% of the registry cases were culture positive, of which 33% had a polymicrobial infection.

**Conclusion:** This sepsis registry is proving to be a key data source for defining the burden of the disease in our community.
Evaluation of nosocomial infection rate during 2013-2014 in Razi Hospital, Ahvaz, Iran

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\textbf{Background:} Nosocomial infections (NIs) are a global medical unresolved issue that imposes much morbidity on the patients and sometimes the health care workers (HCWs), and causes an extra cost of health care that could play an important role in transmitting NIs from patients to other patients and HCWs. The aim of this study was to evaluate the prevalence of NIs in Razi Hospital, Ahvaz, southwest of Iran, during 2013-2014.

\textbf{Methods & Materials:} The present study was a descriptive study, conducted on all the patients who were hospitalized with signs and symptoms of infection after 48 hours of hospitalization, and 600 HCWs in Razi Hospital in Ahvaz, Iran, during 2013-2014 were enrolled. Data about the patients’ site of infection, ward of hospitalization, and type of NI were collected. Data were summarized using descriptive statistical methods and were analyzed by Excel and SPSS 16.0.

\textbf{Results:} The results of the present study showed that the incidence of NIs was low (<2%) in our hospital during 2013-2014. Most NIs was reported in wards of obstetrics and gynecology (OBGYN), orthopedic, Intensive Care Unit (ICU), general surgery, infectious diseases, internal medicine, and Coronary Care Unit (CCU) in decreasing frequency.

\textbf{Conclusion:} Based on these findings, NIs in our hospital had a lower frequency in comparison to the national rates. Training programs related to the prevention of NIs may be one of the reasons for this low frequency in this teaching hospital.
Genetic diversity of common environmental Enterobacteriaceae bacilli in intensive care units of hospitals from central Iran

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Background: Environmental surfaces of intensive care units (ICUs) are suitable for the growth of Gram-negative bacteria. Normally, these strains that are circulating between the environment and patients can cause outbreaks of nosocomial infections among vulnerable patients. In this study, the genetic diversity of common Enterobacteriaceae bacilli family in the environment of the ICUs was evaluated.

Methods & Materials: During a 10-month period, samples were collected from environmental surfaces of ICU departments such as toilets, different surfaces, machineries and equipment, and injectable solutions from 5 hospitals located in Qom, central Iran. Swab samples were transferred on Blood Agar and sub-cultured on MacConkey medium, then suspected colonies were inoculated into the TSI media and differential diagnosis was done by using differential culture media and biochemical reactions for Enterobacteriaceae family. Results were confirmed by using API 20E kit. Genetic diversity was evaluated by using universal primers in ERIC-PCR method. The clonal relatedness of the strains were obtained using GelCompar II software.

Results: A total of 396 swab samples were collected and 1205 colonies were isolated including 194 colonies of Gram-negative bacilli. Totally, 22% of the isolates belonged to Enterobacteriaceae family including Klebsiella pneumoniae (7.21%) and Enterobacter cloacae (2.6%) as the most prevalent. Overall, 14 strains of K. pneumoniae were identified. Band patterns of 2 cluster each with 2 strains had 90% similarity (one clone): two strains were isolated from soap and pillow from hospitals I and III with one month interval, two other strains were isolated from dining table and sink from hospitals IV and V with 4 month interval. Overall, 5 strains of E. cloacae were identified from hospitals IV and V. Band patterns of these strains had less than 50% similarity and were considered as 5 distinct single types.

Conclusion: The results show the diversity of contaminating sources and presence of different circulating clones of E. cloacae. Isolation of similar clones of K. pneumonia from different hospitals is crucial in aspect of controlling the spread of same clone of nosocomial infections.
Antibiotic sensitivity assay of pathogenic microorganisms isolated from selected areas in some primary health centres in Akure Metropolis

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**Background:** Infections acquired in health care settings are among the major causes of death and increased morbidity among hospitalized patients. This study is aimed at isolating and identifying pathogenic microorganisms present in surface of facilities in wards of selected primary health centres and their sensitivity to commercial antibiotics.

**Methods & Materials:** Swab collection of specimen from different surfaces of beddings (pillows and bed sheets), toilet seats, floor and door handles were collected in triplicates to evaluate the hygienic status of the basic primary health centre. This was done or repeated at least two times in two weeks for male, female and children wards of the six basic primary health centres selected in Akure metropolis. A total of seven hundred and twenty (720) swab samples were collected. The samples were analyzed using microbiological standard. Antibiotic sensitivity test was carried out on the pathogenic isolates using these drugs; nitrofurantoin; chloramphenicol; streptomycin; tetracyclin, ampicillin; coltrimoxazole; gentamycin; nalidixic acid ofloxacin while the antifungal sensitivity test was done using Nystatin, Ketocunazole and Fulcin. All the data obtained were subjected to descriptive one way analysis of variance, SPSS version 16, Microsoft window 8 with Duncan New Multiple Range Test as follow up test.

**Results:** A total of seven pathogenic bacteria and two yeasts were isolated. These are *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida albicans* and *Candida dubliensis*. The results showed that the highest bacterial load of 53.33 ± 1.86 cfu/ml was obtained from toilet from maternity ward while the least bacterial load of 1.67 ± 0.33 cfu/ml was obtained from the pillows of pediatric ward. Their sensitivity test to commercial antibiotics showed that *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most resistant bacteria to the antibiotics they were subjected to. Generally, Ofloxacin exert the highest inhibitory effect against all the bacteria, while most of them showed resistance to tetracycline and streptomycin.

**Conclusion:** It could be inferred from the results that pathogenic microorganisms that are resistance to some commonly used drugs can be acquired from these health centres. Therefore, adequate ward hygiene is necessary to reduce hospital acquired infections.
Evaluation of needle stick injuries among health care workers in a teaching hospital

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Background: Needle stick injuries (NSIs) are one of the most important threats for the health care workers (HCWs) in teaching hospitals. HCWs can reduce the rate of NSIs by performing protective procedures. The general purpose of this research was to evaluate the Prevalence of needle sticks injuries among health care workers in Razi Hospital, Ahvaz, during 2014.

Methods & Materials: The present study was a descriptive incidence study conducted on 600 HCWs at Razi Hospital, Ahvaz, Iran. Data about health care workers' type of NSIs, ward, and their activity were collected. Data were summarized using descriptive statistical methods and were processed by SPSS version 16.

Results: Based on the results, nurses were at highest risk of NSIs among HCW groups. In this hospital, 41 cases of NSIs were found. Based on the findings, recapping needles was found in 36.58%, handling needle on tray in 21.95%, suturing in 17.07%, passing needle in 12.19%, transit of disposal needle devices in 4.87%, and dissembling needle devices in 7.31% of cases. Most of the NSIs were reported in wards of general surgery, ICU, emergency, obstetrics and gynecological (OBGYN), orthopedic, operating room, and infectious diseases in decreasing frequency during 2014. The results indicated that recapping the needles was the most risk factor for NSI.

Conclusion: According to the findings of our study, nurses were the most type of HCW sustaining NSI. It seems that training programs related to the prevention of NSIs would be one of the priorities in the Razi teaching hospital.
An integrated and active system based on a multiple PCR method for the surveillance of Carbapenemase producing enterobacteria in an Italian "hub and spoke" large laboratory model

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Background: The spread of entobacteria producing carbapenemases (CRE) is becoming more and more common in the Italian hospitals and many different strategies have been implemented since the last 5 years in order to limit the diffusion of these multidrug resistance germs that represent a life threatening risk for patients that develop invasive infections. Two possible approaches are currently available for the identification of CRE in rectal swabs from potentially colonized patients: the standard workflow based on culture and phenotypical identification of the colonies or the identification of the genes responsible for the carbapenemases resistance. In this study we compared the routine workflow with a rapid and simple to be performed multiplex molecular assay (CARBA geneXpert - Cepheid) for the screening of CRE. The study was performed in a “hub & spoke” large (more than 1,000,000 microbiology tests/year) public laboratory located in the Romagna area (North-East Italy) that serves 7 hospitals with a total of about 4000 beds.

Methods & Materials: A total of 345 rectal swab were collected from patients entering the ER and ICU wards of the “degli Infermi” hospital in Rimini from May to September 2015. The samples were transferred to the Hub lab (some 45 km away from the hospital) by using the scheduled transport system (active from 8:00 am to 2:30 pm, three times from Monday to Saturday) or processed in the spoke lab located in the hospital that is open 24 hours for 7 days in a week. The Hub lab processed the swab upon arrival with both the routine method and the geneXpert assay, the spoke only performed immediately upon reception the geneXpert test. The results obtained by each method used were recorded and the turn around time (TAT) for each individual test/sample was recorded.

Results: The results are summarized in figures 1, 2 and 3. The TAT of geneXpert results was on average within 3 hours whereas the standard method was ranging from 24 to 96 hours.

<table>
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<tr>
<th>Xpert Carbapil</th>
<th>Culture</th>
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<tr>
<td>+VE</td>
<td>7 (KPC, VIM-2, K. pneumoniae, E. cloacae)</td>
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<tr>
<td>-VE</td>
<td>1 (E. cloacae)</td>
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<th>CPE detection</th>
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<td>+VE</td>
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Compartive results obtained with the ruotine workflow and geneXpert assay

Conclusion: The use of geneXpert has proven to be cost effective to rapidly and efficiently identify CRE in a “hub and spoke” laboratory model.
Evaluation of multiple antibiotic resistance (MAR) index and Doxycycline susceptibility of Acinetobacter species among inpatients

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Background: Acinetobacter is contributing to increased morbidity and mortality with its strong propensity to colonize and disseminate among humans and environmental sources.

Methods & Materials: Clinical isolates of Acinetobacter species recovered from inpatients of tertiary care institute in North West India were analyzed retrospectively along with their antibiogram to evaluate in vitro activity of Doxycycline. Isolates resistant to more than three different groups of antimicrobials were considered to be multidrug resistant (MDR). MAR index was calculated and interpreted.

Results: A total 93 isolates of Acinetobacter species were recovered from inpatients, out of which predominant were from urine 47 (50.54%) followed by blood 27 (29.03%) samples. MDR isolates were 57 (61.29%) with majority from urine 35 (61.40%) and blood samples 8 (14.04%). Overall antimicrobial susceptibility pattern revealed Imipenem (75.27%), Meropenem (68.82%) and Doxycycline (68.82%) to be most efficacious drugs whereas in MDR Acinetobacter spp most promising drug was Doxycycline, with highest sensitivity rate (66.67%) followed by carbapenems namely Imipenem (61.40%) and Meropenem (52.63%). MAR index revealed 71 isolates (76.34%) with MAR index greater than 0.2 and 22 (23.66%) less than 0.2. However, three isolates had shown MAR index of 01 (i.e. resistant to all the antimicrobials tested), out of which two were recovered from intensive care unit and one from general surgery ward. Isolates with MAR index greater than 0.2 were mainly isolated from patients admitted in General surgery ward 20 (28.17%), intensive care units 19 (26.76%) and gynecology/obstetrics ward 17 (23.94%). Twenty six multi drug resistance patterns were observed for Acinetobacter species for the nine antimicrobials tested. Resistance to COT, CIP, GEN, AK, A/S, CPM, IMP, MRP (R8) was most frequently observed pattern in 8 (14.04%) of MDR isolates.

Conclusion: MDR Acinetobacter is emerging as predominant pathogen in hospital settings. In current study Doxycycline has exhibited efficacy against MDR Acinetobacter, which can be considered as an alternative therapy to down regulate selective pressure on carbapenems. In low resource settings, antibiogram along with MAR index serve an important epidemiological tool to monitor drug resistance in Acinetobacter species among inpatients as it is becoming more difficult to treat this pathogen due to the restricted pharmaceutical and therapeutic armamentarium.
HSV positivity in bronco-alveolar lavage fluid and clinical outcome in hospitalized patients

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**Background:** Herpes Simplex virus (HSV) is highly prevalent and ubiquitously distributed worldwide. It is responsible for a wide variety of clinical manifestations with a generally benign course in immunocompetent individuals and a worse prognosis in the immunocompromised.

**Methods & Materials:** We studied the clinical, laboratory characteristics and outcome of 49 individuals admitted to the Policlinico Tor Vergata (PTV) of Rome, Italy, from May 2013 to June 2014, whose bronco-alveolar fluid (BALF) samples were tested for the presence of HSV-DNA by qualitative and quantitative PCR using commercially available kits. Statistical significance of the differences was studied by non-parametric tests (U Mann Whitney).

**Results:** Among the 49 patients studied, 19 individuals were HSV-DNA positive and 30 were HSV-DNA negative. The two groups matched according to age, gender and month of BALF sampling. The majority of BALF samples were from critically-ill Intensive Care Units (ICU) patients clinically suspected of ventilator-associated pneumonia (VAP) or from non-ICU patients with suspected pulmonary diseases. Detection of HSV was significantly associated with: cardiac disease (p=0.003 by U Mann Whitney test), length of stay in hospital (p=0.027), disease severity (p=0.003) and risk of death (p=0.009), higher leukocyte (p=0.011) and lower lymphocyte (p=0.0001) counts, lower haemoglobin values (p=0.002) and higher erythrocyte sedimentation rate (ESR) (0.026). Median HSV copy number was 71121 cp/ml in the total sample of HSV-positive patients, but 28132 in the cases who survived and 114110 in the cases who died, albeit this datum did not reach the statistical significance (p=0.90), probably in relation to the low number of patients.

**Conclusion:** Our results confirm that critically ill patients in whom HSV from BALF is isolated, have higher risk of disease severity and fatal outcome mainly because underlying disease and comorbidities and HSV detection appears related to several laboratory findings (leukocyte and lymphocyte counts, haemoglobin values and ESR). Further studies are needed to ascertain whether HSV presence in BALF can be predictive of poorer outcome in critically ill patients.
An outbreak of 19 cases of Serratia marcescens meningitis after spinal anesthesia
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Boufarik Hospital, Algiers, Algeria

Background: Bacterial meningitis is a rare but serious complication of spinal anesthesia (lower incidence of 4.5 cases per 100 000 spinal anesthesia), although individual cases have been reported in the literature, the occurrence of an epidemic remains an exceptional event. Serratia marcescens is an enteric bacterium that causes a variety of infections and nosocomial outbreaks including meningitis. She is mainly encountered in neonatology and neurosurgery. Our goal is to describe the epidemiological, clinical and evolutionary an outbreak of Serratia marcescens meningitis in a private clinic medical and surgical patients who received spinal anesthesia.

Methods & Materials: We report an outbreak of 19 cases of meningitis hospitalized in our department of infectious diseases EPH Boufarik (Algeria) between 18 December 2012 and 15 Jan 2013. All cases underwent spinal anesthesia performed by the same anesthesiologist in same private clinic. A case was defined by the appearance of meningeal symptoms after spinal anesthesia with LCR disturbed.

Results: A total of 19 cases of bacterial meningitis was diagnosed. The attack rate was 14 meningitis / 100 spinal anesthesia. The median age was 33 years (23-77), the interval between onset of symptoms and spinal anesthesia was 4 days (1-6). The most found symptoms were headache, fever, vomiting and neurological disorders. CSF findings: white blood cell count mean 716 cells / mm 3 (32-2000), neutrophil predominance (52.63 %), hypoglycorachie (58 %). S.marcescens was isolated from the CSF of 9 patients and 2 blood cultures. All patients received treatment with ceftriaxone 4 g / day with a minimum of 21 days. The outcome was: favorable for 84.21 % of cases, 2 neurological complications, 2 deaths (10.52 %) and 1 relapse (5.26 %). The epidemiological investigation revealed a failure in hygiene and inadequate products or procedures (hand carriage offending) measures S.marcescens was identified on a catheter collected in a waste for quills.

Conclusion: This epidemic exceptionally large in number of cases and duration. It has initiated us to implementing more strict hygiene measurements and improving the quality of infection control.
Risk factors associated with outbreak of methanol poisoning in southern districts of Ondo State Nigeria, May 2015
A. Adewole
Nigeria Field Epidemiology and Laboratory Training Programme, Abuja, Nigeria

Background: On the 13th of April three people from Irele LGA, Ondo State Nigeria were reported having symptoms of headache, blurring of vision, respiratory symptom and loss of consciousness/death all resulting to death within 24-72 hours of onset. The State ministry of health was alerted and we investigated to characterize the outbreak in terms of time place and person, identify the causative agent, source and mode of transmission and to identify the possible risk factors responsible.

Methods & Materials: A community based case control study was done with a suspect case as any person with headache, blurring of vision and/or blindness, and/or respiratory distress with or without loss of consciousness within 24-48 hours of onset of symptoms beginning from 12th April 2015 in Ode-Irele LGA. Confirmed case as any person who met the ‘suspect case’ criteria with laboratory confirmation of methanol blood and urine levels. Control as any person without the above symptoms residing in Irele LGA of Ondo State. Nineteen cases and 57 controls (1 case to 3 un-matched controls) were interviewed using a semi-structured interviewer administered questionnaire. Data were analyzed using Epi-info statistical software.

Results: A total of 39 cases were line-listed with 29 deaths with the case fatality rate of 74.4%. Mean age was 40.4±12.5 years. Almost all the cases were males 38 (97.4%) and mostly farmers 16 (57.1%). 32 (94.1%) claimed to have consumed local gin prior to development of symptoms. The most common symptoms were blindness 29 (82.9%) and blurring of vision 28 (82.3%). Risk factors for the outbreak were consumption of local gin [17.2;4.6-84.0] and alcohol consumption [24.2;4.0-555.6]. Laboratory findings revealed methanol toxicity in both blood and urine samples as well as toxicology result of the local gin sample.

Conclusion: Local gin contaminated with methanol was the major risk factor for the occurrence of the outbreak.
wgMLST as a standardized tool for assessing the quality of genome assembly data

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**Background:** Continued advances in next-generation sequencing (NGS) technologies is accompanied with the development of many whole genome assembly approaches to convert the small sequences (reads) into larger regions (contigs/scaffolds). However, none of these is perfect. Up to now, genome assembly data is compared under standard statistics (N50, coverage, contig sizes, number bases etc.) and there is no commonly accepted and standardized method for comparison and assessing the assembly data.

**Methods & Materials:** The raw data for S. aureus SA957 (paired end sequencing - SRR497751) produced by Illumina platform have been downloaded from European Nucleotide Archive. Software such as Tadpole, Velvet, CLC genomic workbench, SeqMan NGen for de novo assembly and Bowtie2, BWA and CLC genomic workbench for mapping to reference have been used under default options to produce contigs/consensus. The assessing of the quality of genome assembly have been performed with using wgMLST implemented in SeqSphere software (Ridom). Concordance of genome assembly data was estimated with Rand index.

**Results:** Seven genomes have been assembled using de novo and reference mapping methods. The basic statistics of de novo assembled data showed that CLC genomic workbench tool gave the best assembly sequence. Statistic parameters of Tadpole assembly were less well in comparison with other (Table 1).

wgMLST analysis based on 2787 genes was performed on seven assembled genomes. As a result, CLC, Velvet and SeqMan Ngen assembly allowed to determine more than 2500 genes while Tadpole assembly with average contig length 1378 bp could identify 1560 out 2787 genes (table 2). Reference mapping assembly revealed high concordance (98-99%) between results. Minimum spanning tree clustered reference mapping results (picture1).

**Conclusion:** A standardized gene-by-gene wgMLST approach allows assessing not only the quality but also quantitative estimation of genome assembly data. Based on this approach the genomes assembled with different software can be compared and clustered to find approaches that give similar results. wgMLST allows to find the discrepancies on gene level as well.
Dependence of the genetic relatedness between isolates on the size of sequencing genes in MLST analysis

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Background: Multilocus-sequence typing (MLST) based on sequencing internal part of well-chosen housekeeping genes/loci has become the method of choice for typing of epidemiologically important strains.

Methods & Materials: 5 MLST schemes have been used for typing of 3496 of E.coli genomes – whole gene MLST (645-2415bp), standard MLST(452-536bp) and 3 modified MLST: +100bp MLST (552-636bp), -100bp MLST (352-436bp), -200bp MLST (252-336 bp). Clonal complexes (CC) were defined as groups with six identical alleles and minimum 5 genotypes. Discriminatory power and concordance were estimated based on Simpson and adj.Wallace indices.

Results: In 3061 cases, genes/alleles have been determined successfully for all five MLST schemes. Discriminatory index for each genes (alleles) and combination of 7 genes (alleles) is presented in Table 1.

<table>
<thead>
<tr>
<th>MLST</th>
<th>adk</th>
<th>fumC</th>
<th>gyrB</th>
<th>icd</th>
<th>mdh</th>
<th>purA</th>
<th>recA</th>
<th>7genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>geneMLST</td>
<td>0.898</td>
<td>0.954</td>
<td>0.962</td>
<td>0.962</td>
<td>0.924</td>
<td>0.931</td>
<td>0.934</td>
<td>0.982</td>
</tr>
<tr>
<td>+100bpMLST</td>
<td>0.898</td>
<td>0.922</td>
<td>0.933</td>
<td>0.924</td>
<td>0.918</td>
<td>0.88</td>
<td>0.863</td>
<td>0.974</td>
</tr>
<tr>
<td>standardMLST</td>
<td>0.887</td>
<td>0.918</td>
<td>0.928</td>
<td>0.92</td>
<td>0.911</td>
<td>0.868</td>
<td>0.85</td>
<td>0.971</td>
</tr>
<tr>
<td>-100bpMLST</td>
<td>0.885</td>
<td>0.917</td>
<td>0.925</td>
<td>0.905</td>
<td>0.908</td>
<td>0.773</td>
<td>0.76</td>
<td>0.971</td>
</tr>
<tr>
<td>-200bpMLST</td>
<td>0.879</td>
<td>0.902</td>
<td>0.918</td>
<td>0.901</td>
<td>0.865</td>
<td>0.736</td>
<td>0.312</td>
<td>0.969</td>
</tr>
</tbody>
</table>

All MLST schemes concordance calculated with adj.Wallace index is presented in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>geneMLST</th>
<th>+100bpMLST</th>
<th>standardMLST</th>
<th>-100bpMLST</th>
<th>-200bpMLST</th>
</tr>
</thead>
<tbody>
<tr>
<td>geneMLST</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>+100bpMLST</td>
<td>0.678</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>standardMLST</td>
<td>0.603</td>
<td>0.890</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>-100bpMLST</td>
<td>0.592</td>
<td>0.872</td>
<td>0.979</td>
<td>1.000</td>
<td>0.927</td>
</tr>
<tr>
<td>-200bpMLST</td>
<td>0.549</td>
<td>0.809</td>
<td>0.908</td>
<td>0.927</td>
<td>0.927</td>
</tr>
</tbody>
</table>

Based on CC parameters there have been 44 CC with 1951, 23CC with 1883, 17CC with 1720, 12 CC with samples and 4 with 1544 samples in gene MLST, +100bp MLST, standard MLST, -100bp MLST and -200bp MLST respectively.

As an example of transformation of Cluster Complex according to the sequencing size is shown in Figure 1

Conclusion: Artificial methods of subspecies typing gives a relative picture of the genetic relationship and clonal structure of microorganisms.
In silico comparison of different PFGE and wgMLST

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Background: Pulsed field gel electrophoresis (PFGE) is acknowledged to be the ‘gold standard’ for the typing of strains of a number of bacterial species, including E. coli, and is used widely in clinical settings (van Belkum A., 2007).

Methods & Materials: In silico PFGE analysis of 138 complete E.coli genomes using classical XbaI and 5 other enzymes (Sse8647I, Apal, AclN, SrfI and SdiI) have been performed by Geneious (Biomatter). Images with gel pattern have been analyzed by TotalLab 1D (Nonlinear Dynamics) to produce band matrix. wgMLST scheme with 2216 loci have created with SeqSpere (Ridom). Discriminatory power and concordance between different PFGE and wgMLST have been estimated based on Simpson and adj.Rand and Wallace indices.

Results: 138 genomes of E.coli have been used to produce different PFGE and wgMLST patterns. Sites of restriction, band (loci) numbers and discriminatory power are presented in Table1.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Recognition sequence</th>
<th>Median of band number</th>
<th>95%CI</th>
<th>#different types</th>
<th>Discriminatory index</th>
<th>Confidence interval (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XbaI</td>
<td>T^CTAGA</td>
<td>39</td>
<td>27-51</td>
<td>131</td>
<td>0.999</td>
<td>[0.998 - 1.0]</td>
</tr>
<tr>
<td>Sse8647I</td>
<td>AG^GWCCT</td>
<td>73</td>
<td>57-104</td>
<td>131</td>
<td>0.999</td>
<td>[0.998 - 1.0]</td>
</tr>
<tr>
<td>Apal</td>
<td>GGGCC^C</td>
<td>77</td>
<td>62-130</td>
<td>132</td>
<td>0.999</td>
<td>[0.998 - 1.0]</td>
</tr>
<tr>
<td>AclN</td>
<td>A^CTAGT</td>
<td>79</td>
<td>53-95</td>
<td>129</td>
<td>0.999</td>
<td>[0.998 - 1.0]</td>
</tr>
<tr>
<td>SrfI</td>
<td>GCCC^GGGC</td>
<td>51</td>
<td>41-65</td>
<td>131</td>
<td>0.999</td>
<td>[0.998 - 1.0]</td>
</tr>
<tr>
<td>SdiI</td>
<td>GGCNNNN^NGGCC</td>
<td>38</td>
<td>31-66</td>
<td>129</td>
<td>0.999</td>
<td>[0.998 - 1.0]</td>
</tr>
<tr>
<td>wgMLST</td>
<td>2216 loci</td>
<td>-</td>
<td>-</td>
<td>129</td>
<td>0.999</td>
<td>[0.998 - 1.0]</td>
</tr>
</tbody>
</table>

The concordance between different PFGEs and wgMLST calculated on cluster complex is presented in Table 2.

<table>
<thead>
<tr>
<th>Enzymes for PFGE</th>
<th>adj.Rand</th>
<th>Wallace wgMLST -&gt;PFGE</th>
<th>Wallace PFGE -&gt; wgMLST</th>
</tr>
</thead>
<tbody>
<tr>
<td>XbaI</td>
<td>0.809</td>
<td>1</td>
<td>0.737</td>
</tr>
<tr>
<td>Sse8647I</td>
<td>0.816</td>
<td>0.998</td>
<td>0.749</td>
</tr>
<tr>
<td>Apal</td>
<td>0.818</td>
<td>0.998</td>
<td>0.75</td>
</tr>
<tr>
<td>AclN</td>
<td>0.821</td>
<td>1</td>
<td>0.751</td>
</tr>
<tr>
<td>SrfI</td>
<td>0.808</td>
<td>0.998</td>
<td>0.738</td>
</tr>
<tr>
<td>SdiI</td>
<td>0.805</td>
<td>1.0</td>
<td>0.741</td>
</tr>
</tbody>
</table>

Conclusion: PFGE using different restriction enzymes, which have different site restriction and produce different number (27-130) of band, have not shown the advance in discriminatory power and concordance with wgMLST.
Hemophagocytic Lymphohistiocytosis (HLH) secondary to infections- Experience at a tertiary care centre

S. Deme
Nizams institute of medical sciences, Hyderabad, Telangana, India

**Background:** Hemophagocytic Lymphohistiocytosis (HLH) is a rare potentially life-threatening disorder characterized by immune dysregulation, overwhelming immune activation and inflammation. This condition can occur as primary or secondary to infections, autoimmune diseases and malignancies. HLH secondary to infections is an important clinical entity especially in tropical countries. We report our experience of HLH from our hospital.

**Methods & Materials:** This is a retrospective analysis of clinical information of patients presented to our hospital between March 2012 and November 2015. All fulfilled the revised criteria of HLH 2004.

**Results:** Total 5 cases were segregated with secondary HLH diagnosis. The mean age at diagnosis was 34 years (with a range of 28 to 50 years). All were males. All patients presented with prolonged fever, hepatomegaly and/or splenomegaly. All of them had at least a bi- or trilineage cytopenia, elevated liver enzymes, hyperferritenemia and hypertrygliceridemia. Four out of five patients had hypofibrinogenemia and hemophagocytosis in bone marrow.

The cause of HLH in our series included scrub typhus-1, military tuberculosis-1, enteric fever-1, HIV infection-1 and septicemia-1. All of them received treatment for underlying primary infection along with supportive care. One patient received steroids for HLH. Four patients expired due to multi-organ dysfunction and one recovered.

**Conclusion:** HLH associated with infections, may resolve with treatment of the underlying infection, and their early recognition is important as they may mimic malignancy. All patients meeting the criteria for HLH should undergo initial tests to diagnose the underlying infecting organism. The mortality rates in adults are high due to delayed diagnosis and multiorgan involvement. A high index of suspicion especially in patients with unresolved fever and persistent cytopenias and elevated ferritin helps in early diagnosis, prompt initiation of treatment and improved outcome.
Hemophagocytic lymphohistiocytosis (HLH) secondary to tropical infections-experience at a tertiary care centre
1Nizams institute of medical sciences, Hyderabad, Telangana, India, 2Nizam's Institute of Medical Sciences, Hyderabad, India, 3Nizams institute of medical sciences, Hyderabad, India

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The hidden epidemic: MERS-CoV-related stigma observations from the field, Qatar 2012-2015


1Supreme Council Health, doha, Qatar, 2Supreme council of Health, Doha, Qatar, 3Sidra Medical and Research Center, Doha, Qatar, 4National Institute for Public Health and the Environment, Bilthoven, Netherlands, 5Supreme Council Health, Doha, Qatar, 6Erasmus Medical Center and Center for Infectious Disease Control, Bilthoven, Netherlands

Background: As most of the attention is focused on understanding how MERS-CoV is being transmitted to develop effective control measures, social stigma has not been assessed. Previous experiences with SARS, H1N1, and Ebola outbreaks showed that fear and stigma can have a serious impact on the disease control. This study sought to document features and implications of MERS-Cov-related stigma in Qatar 2012-2015.

Methods & Materials: Investigation records of all MERS-CoV reported human/animal cases were reviewed along with the field observations of the MERS-CoV investigation team to elicit stigma-related ideas and behaviors in Qatar during the period 2012-2015.

Results: Two types of stigma were observed; Human-related and camels-related. Features of stigma included: the denial of having the disease, rejection of IPC measures, escape or discharge against the medical advice, poor compliance with the medical isolation, getting rid of the suspected animals, and escape of camel-workers from the MERS affected the barns.

Stigma Implications: Early reporting and detection of suspected cases was negatively affected as people tend to withhold information about their relationship with camels or having a history of contact with a confirmed/suspected case, hindering identification and investigation of the possible human/animal contacts. Since most of the vulnerable people had co-morbidities, serious implications were observed because of the delayed reporting to hospitals which might yield poor outcome.

Conclusion: Preserve the confidentiality of the human/animal cases is paramount to maintain trust along with the adoption of effective patient education and counseling. Fostering the early engagement of communities, the transparent sharing of information with the public, and the implementation of One-Health Approach can help mitigate stigma.
Comparison of severity of sepsis with various biochemical parameters

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Background: Vitamin D is an immuno-modulator in humans and its deficiency has been reported in patients in sepsis and may contribute to multi-organ dysfunction and nosocomial infections seen in them. Vitamin D is derived from cholesterol stored in the body by the action of sunlight. Procalcitonin has been a widely accepted parameter for severity of sepsis. OBJECTIVE: To measure vitamin D levels in sepsis patients and to compare it with various mortality indicators used in critically ill patients in sepsis like SAPS II score, Charlson Comorbidity Index, procalcitonin and to find any association between the lipid profile of the patient with vitamin D levels and severity of sepsis calculated by mortality, ICU and hospital length of stay and duration of mechanical ventilation and to match them with vitamin D levels of age and sex matched healthy patients.

Methods & Materials: IEC permission was obtained to carry out this study. From 96 sepsis patients, plasma vitamin D (measured within first 24 hours after ICU admission as standard of care), demographic data (age, sex), Simplified Acute Physiology Score (SAPS II), mortality were recorded. Co-morbidities were assessed using the age-unadjusted Charlson Comorbidity Index. Procalcitonin levels were noted for patients along with lipid profile, whenever available. Vitamin D values were recorded from age and sex matched non-sepsis OPD patients, taken as controls.

Results: The average SAPS II score in sepsis patients was 43.49. Correlation of vitamin D with age, procalcitonin levels (fig 1), ICU length of stay (LOS) (fig 2), Hospital LOS and mortality showed a negative correlation. Procalcitonin levels had a positive correlation with SAPS II score, days of Mechanical Ventilation (MV), ICU LOS and mortality. The average vitamin D level in patients of sepsis in our study was 15.38 ng/dl and that of controls was 41.11 ng/dl (fig 3) and Vit D had no significant correlation with lipid profile.

Conclusion: Deficient levels of vitamin D has a possible role in sepsis. Hence supplementation of vitamin D might have a beneficial role in sepsis management and overall outcome. Further interventional studies with larger sample size and supplementation of vitamin D is required to substantiate the findings.
Dengue: Mathematical modelling of cytokine levels in the evoultion of severity
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¹University of Colombo, Colombo, Sri Lanka, ²University of Sri Jayawardenapura, Sri Lanka, Colombo, Sri Lanka

Background: Dengue causes considerable morbidity and mortality in Sri Lanka. Immune mediated and cytokine related factors contribute to its evolution from an asymptotic infection to severe forms of dengue. Previous studies have analysed the association of individual cytokines with clinical disease severity. In contrast, we have viewed this evolution to severe dengue as the behaviour of a complex dynamic system. We therefore analysed the combined effect of multiple cytokines that interact dynamically with each other in order to generate a mathematical model to predict the occurrence of severe dengue. We expect this to have predictive value in detecting severe cases and improve outcomes.

Methods & Materials: We analysed data on 11 adult patients with dengue fever (DF) and 25 patients with dengue haemorrhagic fever (DHF) recruited from the Colombo South Teaching Hospital, Sri Lanka. Platelet activating factor (PAF), sphingosine 1- phosphatase (S1P), IL1β, TNFα and IL10 were used as the cytokine parameters for the model. Hierarchical clustering was used to detect factors that correlated with each other. Their interactions were mapped using Fuzzy Logic mechanisms with the combination of Hamacher and OWA operators.

Results: Clustering indicated that S1P and IL1β levels were associated with each other. Since, PAF, IL-10 and TNF-α have shown to associate with severe dengue, they were combined together by allocating these cytokines a higher prominence in the model. Operator value below 0.3 in the overall model correctly predicted development of DHF with 76.6% accuracy. A region of ambiguity was detected in the model for the value range 0.35 to 0.55. However, in six instances patients with DHF indicated operator values above 0.6 and in four instances, patients with DF showed operator values below 0.35. The accuracy of this model in predicting severe dengue was 76.19% at 96 hours from the onset of illness, 75% at 108 hours and 74.07% at 120 hours.

Conclusion: The results show a robust mathematical model that explains the evolution of dengue infection to its serious forms. This model should be further improved by including additional parameters and be validated on other data sets.
Engineering of measles virus to target cancer cells, an attempt
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1Jawaharlal Nehru University, New Delhi, New Delhi, India, 2Jawaharlal Nehru University, New Delhi, India

Background: Regardless of general perception as potentially dangerous pathogens, viruses have been exploited and used as vaccine agents or as carriers for gene therapy. Similar positive effects have been observed in case of cancer patients getting infected with viruses, where infection has resulted in temporary tumor regression. Hence, the development of a recombinant virus that selectively infects and kills cancer cells can be a promising anti-cancer tool in near future. Here we made an attempt to generate an oncolytic virus using Measles viral genome (Edmonston strain) backbone and to further arm this recombinant virus with non viral genes of known anti-proliferative activity to enhance its antitumor activity.

Methods & Materials: Genes encoding Nucleoprotein (N) and Phosphoprotein (P) of Measles virus were cloned into expression vector pcDNA(3.1+). HEK293 cells were stably transfected with viral N and P constructs to generate a packaging cell line for the recovery of recombinant virus. Gene encoding viral L polymerase (RNA-dependent RNA polymerase) was cloned in pcDNA(3.1) and co-transfected with the Measles viral full-length genome construct (Addgene #58748) in packaging cell line to enable the generation of viral negative sense genome. For arming of the virus, the gene encoding a pro-apoptotic protein BNiP3 of human origin will be inserted into the recombinant Measles viral genome upstream of Matrix gene.

Results: Co-expression of measles virus N and P proteins in packaging cell line (HEK293-N/P) was confirmed by IFA staining (Fig1). Gene encoding L polymerase construct was generated (Fig 2). Expression of viral genes in packaging cells transfected with full-length viral genome was confirmed at transcript and protein levels. Further, expression of viral genes in Vero cells infected with the lysates recovered from packaging cells transfected with the recombinant viral genome confirmed its replication competency (Fig 3).

Expression of Nucleoprotein and Phosphoprotein of Measles virus in the packaging cell line

Generation of L polymerase plasmid construct of Measles virus

Infection of Vero cells with recombinant Measles virus generated in the packaging cell line

Conclusion: The components required for the construction of an oncolytic Measles virus were successfully generated. Studies are ongoing to rescue the recombinant virus from packaging cell line and to further arm the recombinant Measles virus with BNiP3 and validate its anti-tumor activity. This study is aimed towards finding the therapeutic potential for an infective virus particle reprogrammed to emerge as an alternative to conventional anti-cancer therapy.
Prevalence of otitis media and its hearing loss in children of South Indian population

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\textsuperscript{1}Institute of Genetics and Hospital for Genetic Diseases, Hyderabad, Telangana State, India, \textsuperscript{2}Institute of Genetics and Hospital for Genetic Diseases, Hyderabad, India, \textsuperscript{3}MAA Research Foundation, Hyderabad, India, \textsuperscript{4}Institute of Genetics and Hospital for Genetic Diseases, Hyderabad, India

**Background:** Otitis media (OM) refers to bacterial infection or an inflammation of middle ear cleft in younger children than adults. The effusion of fluids in the middle ear or pathological changes in the tympanic membrane or ossicles leads to hearing loss. The aetiopathogenesis of OM is due to the involvement of multiple factors such as demographic, genetic, environmental and other health related factors like infections, allergy, asthma, eustachian tube dysfunction, cleft palate, and adenoid hypertrophy etc., Therefore, the present study aimed to determine the prevalence of OM subtypes and its association with hearing loss in children of South Indian population.

**Methods & Materials:** All the 896 patients with otitis media seen in MAA ENT Hospitals, Hyderabad, Telangana State, from 2010 - 2014 constituted the study subjects. The patients whose age ranged from 1-15 years with symptoms such as otalgia, otorrhea/ inattentiveness and clinical examination which showed fluid behind intact tympanic membrane supported by 'B' type/high gradient tympanometry constituted the study subjects. The chi-square test was used for comparing the proportions of categorical variables by using Statistical Package for Social Sciences, PASW STATISTICS 18.0 software (SPSS Inc., Chicago, IL, USA).

**Results:** Out of 896 OM patients, Acute suppurative otitis media(ASOM) were 15.5\%, chronic suppurative otitis media(CSOM) were 65.3\% and otitis media with effusion(OME) were 19.2\% with male preponderance of 1.8:1. With regard to seasonal variability, the occurrence of OM was more during winter. The occurrence of ASOM and CSOM was more unilateral except in OME which showed bilaterality. It was also observed that more prevalence of sensorineural form is noticed in ASOM and OME while mixed form in CSOM.

**Conclusion:** CSOM is one of the most common inflammatory disorders of middle ear and has an important health concern in children. Continuous efforts in the treatment of hearing impairment rehabilitation are to be taken for the proper management of OM. Therefore, OM is a condition of serious pediatric concern, research on the genetic aspects may help to understand the underlying mechanisms for formulating better therapeutic and preventive strategies.
Association of Histone acetylation and DNA repair genes of Leishmania donovani effect the cytotoxicity of Ultraviolet radiation

A. mishra¹, I. khan¹, P. jha¹, P. Das², K. K. Sinha¹
¹NIPER, Hajipur, Hajipur, India, ²RMRI, Patna, India

**Background:** Remodelling of chromatin affects important DNA processes such as replication, transcription, recombination, repair etc. Recent studies have shown the role of histone modifying enzyme in proliferating cell nuclear antigen (PCNA) degradation upon exposure to UV. Also, H2AX phosphorylation is an established marker for DNA damage and it is essential for the recruitment of DNA repair enzymes. In case of *Leishmania donovani*, it is more important because the parasite lives inside the host macrophage under very oxidative enviroment. Hence it is prone to lot of DNA damage and depend upon various survival strategies.

**Methods & Materials:** In the present study, the acetylation of leishmanial cells was modulated by treating the cells with HAT activator or HAT inhibitor. Treated cells were subjected to UV irradiation and cell survival was measured and compared with that of untreated cells.

**Results:** The HAT treated cells showed higher sensitivity towards UV rays in compare to its untreated counterpart. Our experimental observation indicates histone modifications may play an important role in the regulation of DNA repair pathway in *Leishmania donovani*.

**Conclusion:** This indicates role of Histone modification in the recruitment of DNA repair enzymes in *L. donovani* after DNA damage. Also, the chromatin compactness can be directly correlated with the damage induced by UV as HAT opens the chromatin structure making it more susceptible.
Background: Sudden death is rare in the paediatric age group. Hand, foot and mouth disease (HFMD) is caused by enteroviruses such as Coxsackie virus A16 (CVA16) and Enterovirus 71 (EV71). The severe form of this disease can lead to death due to neurological and cardiopulmonary complications. The diagnostic hallmarks are oral ulcers and macular-papular or vesicular rash on the hands and feet. Most deaths occur in hospitals with the child exhibiting the typical manifestation of the disease. This case aims to describe a fatal case of HFMD with minimal oral and skin manifestations. The lack of awareness of this phenomenon could lead to a misdiagnosis at autopsy.

Methods & Materials: Case Report: A four-year-old girl was brought to the Hospital after suddenly becoming unresponsive at home. She had a history of fever and lethargy for three days prior to the event. Four other children in her neighbourhood had fever with vesicular eruptions at the palm and soles. The children, including this patient, were diagnosed to have HFMD at a local clinic; the other children had recovered without complications.

Results: Autopsy revealed a well-nourished female child with appropriate build for age. There were no vesicles or other lesions seen at the characteristic places. However, close examination with a magnifying glass showed a few punctate, colourless, sub-epidermal vesicles measuring 1 to 2 mm, at the right palm and sole. Internal examination revealed prominent nodularity at the oro- and hypopharynxes. The lungs were markedly congested and oedematous. The brain, heart, liver and kidneys were grossly unremarkable. Histopathology of the lung showed pneumonic changes. Oedema with increase in macroglia and astrocytic proliferation were seen in the cerebral tissue, but no lymphocytic infiltration is evident. The oro- and hypopharynx nodularity was due to mucosal lymphoid follicle hyperplasia. Enterovirus EV71 was detected by polymerase chain reaction in samples from the lung, cerebrospinal fluid and serum. The cause of death was given HFMD complicated by pneumonia.

Conclusion: HFMD may exhibit minimal oral and skin manifestations; this is not necessarily associated with a good outcome. At autopsy, proper history, physical examination and appropriate investigations are essential for arriving at the right diagnosis.
The outcome of cancer treatment is independent of baseline HIV viral load and CD4+ cell count status: a pilot study from South Africa

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**Background:** Increased life years of HIV positive patients has increased the number of HIV related chronic conditions and cancers. On the other hand, chemotherapy and radiotherapy remain key treatment methods for cancer, which could exacerbate the immunosuppression status. There is limited data on the outcome of cancer therapy in HIV positive patients on HAART in South Africa.

**Methods & Materials:** This prospective study was based on 34 cancer patients (31 female [mean: 43 years] and 3 males [mean: 36 years]). The majority had cervical cancer (22), followed by Kaposi sarcoma (7), ovarian cancer (3) and one each with vulvar and choriocarcinoma. The study population was referred to DGMAH from Limpopo, North West and Gauteng provinces. HIV viral load (HIV VL), CD4+ and CD8+ cell count were performed through NHLS.

**Results:** Data on follow-up of 6 to 12 months post cancer therapy was available for 10 patients from the gynaecology outpatient department. The mean age was 42.4 years (range: 25 to 60). Of the 10 patients, 50% were HAART experienced at recruitment with lower than detectable HIV VL and average CD4+ cell count of 411.6 cells/ul. The other 50% of patients were HAART naive with an average HIV VL of log 4.27 and CD4+ cell count of 267 cells/ul. All patients with a detectable HIV viral load attained a lower than detectable status by the end of cancer therapy. Seven patients experienced a significant (p= 0.0001) reduction of CD4+ cell count (the mean was from 261.67 to 41 cells/ul) by the end of cancer therapy. Seven patients achieved complete remission of cancer without complications after an average 8.5 (range: 6 to 12) months of follow-up. Complications were noted in two patients at the end of cancer therapy. One patient was lost to follow-up.

**Conclusion:** Although the sample size was limited, this study demonstrated that the outcome of cancer treatment appears to be independent of baseline HIV viral load and CD4+ cell count.
Effects of dietary supplementation of Lactobacillus plantarum probiotics from corn slurry on growth performance, gut morphometry and profile of the intestinal microbial flora of Clarias gariepinus fingerlings

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Background: Aquaculture growth is considered to be more rapid than all other animal food sectors. The increase in the intensification and commercialization of aquaculture production has been associated with need to overcome challenges such as development of appropriate feedstuffs as well as improvements in water-quality management. Many feed ingredients are not fully digested by fish, however, the addition of probiotics to feed can enhance digestibility of feed components. Hence, this Study was conducted to evaluate the effects of Lactobacillus plantarum on growth performance, gut morphometry and profile of the intestinal microbial flora of Clarias gariepinus fingerlings.

Methods & Materials: A total of 150 Clarias gariepinus fingerlings (2.35±0.48g/fish) were randomly allocated to five treatments, with three replicates of ten fish. Lactobacillus plantarum isolated from corn slurry was cultured using standard procedures. Five isonitrogenous diets were formulated at 35% crude protein (T₀, T₁, T₂, T₃ and T₄) with L. plantarum at inclusion levels of 0%, 0.5%, 1.0%, 1.5% and 2.0% respectively using Pearson Square method. Fish were fed twice daily at 09.00 and 17.00 hours at 5% body weight per day for 12 weeks.

Results: Results showed that weight gain and specific growth rate of Clarias gariepinus increased with increasing level of probiotics. The lowest Mean Weight Gain, and Specific Growth Rate, was recorded in T₁, while the highest was obtained in T₄. Feed conversion ratio was marginally lower in T₄ (1.97) when compared with other treatments. Villi length was statistically similar in T₀, T₁ and T₃, but was significantly higher (P<0.05) in T₄. Villi width was however significantly high in T₁, T₂, T₃ and T₄. The highest cryptal depth was recorded in T₄ which was significantly higher, while the least value was recorded in T₁. T₄ gave the highest enterobacteriaceae count while the least count was recorded in T₀.

Conclusion: From these results, the use of Lactobacillus plantarum as a supplement in diets may be useful in improving growth performance, through increased absorptive capacity as well as increasing the intestinal microbial count in the gastro-intestinal tract of cultured C. gariepinus fingerlings.
Improvement of DNA extraction from human biopsies for a microbiome metagenomic approach

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Background: The last few years have seen an accelerated expansion of microbiome studies rendering a clear picture of its major role in human health and disease. Several international studies have shown that the human gut microbiome consists of four enterotypes; however, these studies have not included Latin American population and have not analyzed differences according to diet that can be important microbiome modifiers. On the other hand, few studies have addressed the eye microbiome and microbiome diversity is unknown. In Mexico, Irritable Bowel Syndrome (IBS) and Dry Eye Syndrome are very common disorders and it has been considered that the microbiome is implicated in both of them. Therefore, it is highly important to study the microbiome in these disorders in our population.

Methods & Materials: Microbiome composition will be determined by next generation sequencing. Sample standardization is reported herein: DNA extraction was performed from 4 colonic and 1 conjunctiva biopsies, collected at the HGM and the IOCV, respectively. The samples were preserved in RNA later (Ambion) at room temperature and were processed within 3 h of collection. Tissue disruption was conducted with proteinase K enzymatic treatment in buffer AL (QIAamp DNA mini Kit, Qiagen) at 56°C for 90 min. The extraction was carried out according to the manufacturer’s protocol. The obtained DNA was analyzed with NanoDrop2000 (Thermo Scientific) for DO 260/280nm ratio and for DNA concentration estimation. Also, an agarose gel electrophoresis was run to visualize the DNA integrity.

Results: This protocol allowed us to obtain an average DNA quantity of 6µg from the colonic biopsies and 4µg per conjunctiva-biopsy with a DO 260/280nm greater than 1.8 and no visible DNA degradation base on literature.

Conclusion: The data suggest that this protocol is suitable for obtaining genomic DNA from colonic and conjunctival biopsies for next generation sequencing.

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Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) might be an independent marker for anticipating scar formation in children with acute pyelonephritis

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**Background:** Urinary tract infections (UTIs) are the most serious common bacterial infections among young children. It may affect kidneys that classified as acute pyelonephritis (APN) and may lead to renal parenchymal involvement and scarring with high prevalence rate (15-60%) among children. This study aimed to assess the urinary concentrations of neutrophil gelatinase-associated lipocalin (NGAL) in patients with APN to diagnose those with potency to scar formation.

**Methods & Materials:** Children who were admitted with a diagnosis of APN were enrolled and divided into 2 groups; APN with scar and APN without scar. Urinary levels of NGAL and its ratio to creatinine (Cr) levels were measured in the acute phase of infection. A receiver operating characteristic (ROC) curve was generated to allow calculation of cut-off values.

**Results:** Sixty-one children were enrolled across the 2 groups: group 1 consisted of 16 patients (all female); group 2, 38 children (36 female and 2 male). Urinary levels of NGAL were significantly higher in APN with scar than in APN without scar (p = 0.037). For comparison of groups 1 and 2, the cut-off values were measured as 7.32 ng/mL, sensitivity; 81.3% and specificity; 66%.

**Conclusion:** Evaluation of urinary NGAL levels may help us to identify children with APN who are at risk of developing renal scarring.
Elucidation of the role of non-structural viral protein (W) of Newcastle disease virus
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Background: Newcastle disease (ND) is a highly contagious disease of birds infecting more than 250 avian species across the world. In India, ND is an economically important and endemic poultry disease. ND is mostly fatal in infected flocks and the currently available vaccines are ineffective. It is caused by Newcastle disease virus (NDV) belonging to family Paramyxoviridae. NDV is an enveloped virus carrying a negative-sense, single-stranded RNA genome with six genes arranged in tandem coding for six structural proteins: nucleocapsid (NP) protein, phosphoprotein (P), matrix (M) protein, fusion (F) protein, hemagglutinin-neuraminidase (HN) protein and large polymerase (L) protein. Additionally NDV expresses two non-structural (NS) proteins, V and W, by co-transcriptional (mRNA) editing of P gene via polymerase stuttering mechanism. Insertion of a single, non-templated G residue results in V protein and insertion of two G residues leads to W protein. These two NS proteins share common N-terminal with P protein and vary at their C-terminal. The NS proteins are not packaged in the virion but expressed only when the virus is actively replicating in the host. While the role of V protein has been extensively studied and reported to be anti-interferon, the function of W protein remains elusive. Our current study is attempted to answer the following questions: Could W mRNA and/or W protein be key factor(s) for viral replication and transcription and/or help evade host immune response?

Methods & Materials: We performed sequence analysis of W protein by bioinformatics. We conducted localization and mutation studies on W protein.

Results: Our preliminary study on sequence analyses of W protein revealed a stretch of basic amino acid residues in the C terminal indicating probable nuclear localizing signal. Our subcellular localization studies confirmed localization of P and V in cytoplasm while W protein predominantly localized in nucleus. By mutation studies we have identified the amino acid residues responsible for nuclear localization of W protein.

Vero cells were transfected with HA tagged W, a nonstructural protein of Newcastle Disease Virus. Cells were fixed and permeabilized 1 day later. Confocal image here shows the nuclear localization of W. Nuclei are stained with DAPI in blue.

Vero cells were transfected with HA tagged V, a nonstructural protein of Newcastle Disease Virus. Cells were fixed and permeabilized 1 day later. Confocal image here shows the expression of V in cytoplasm. Nuclei are stained with DAPI in blue.
Alignment of amino acid sequence at the C-terminal domain of W protein showing the basic amino acid rich residues (Bolded and underlined) as the probable nuclear localizing signal.

**Conclusion:** The stretch of basic amino acid residues at the C terminal region of W protein is important for its localization into the nucleus. Our future direction is towards understanding the role of W protein in the nucleus.
Community acquired Staphylococcus aureus infection in previously healthy neonates in Argentina

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Background: Community-Acquired S aureus (CA-Sa) resistance are changing in the last ten years in Argentina, increasing methicillin-resistant S aureus (MRSA) strains. First case in our neonatal unit was diagnosed in 2006. The objective was analyze clinical, epidemiological, microbiological and outcome features of neonates with CA-Sa infection

Methods & Materials: Prospective observational study. We included previously healthy patients (p) ≤ 30 days of age admitted in the neonatal unit with CA-Sa infection from 2006 to 2014. We defined CA-Sa infection based on CDC guidelines.

Results: We included 37 healthy neonates with CA-Sa infection. Twenty nine (78%) were born by vaginal labor. Mean gestational age was 39 weeks (r: 35-41), weight 3279 grams (r: 2500-4450). Patients had less than 3 birth hospital days in 95% of cases. Contact with parental soft tissue infections were present in 38%, 65% of them were associated with maternal furunculosis. Nineteen p (51%) were female. Mean age at admission was 18 days (r: 4-30). Thirty four p (92%) had skin and soft tissue involved at admission, with cellulitis, bullous impetigo, pustulosis, chest, facial/neck, inguinal and groin abscesses and mastitis. Fifteen p (40%) also had invasive disease: sepsis, omphalitis, osteoarthritis, orbital cellulitis, cerebral abscess, meningitis, liver abscess and necrotizing pneumonia. Only 3p with invasive disease (osteoarthritis: 1p, otitis: 1p and pleuropulmonary infection: 1p) didn’t have skin and soft tissue manifestation at admission. Positive culture was obtained in 33p (89%) from purulent effusion, and 4 (11%) from blood culture alone. Between 37 CA-Sa infections, 27 (72%) were MRSA and 11% were clindamycin resistant too. All patients were treated with antibiotics and in 24p (65%) surgical drainage was performed. Thirty four p (92%) received sequential parenteral –oral treatment with a mean duration of 21 days (r: 1-60d). Median hospital stay was 12 days (r: 4-60). One patient (2, 7%) died because of sepsis and necrotizing pneumonia.

Conclusion: Epidemiology of CA-Sa infection is changing in the newborn period with increasing CA-MRSA strains in the last 8 years in Argentina. We have to consider it for the empirical antibiotic treatment especially in those p coming from the community with skin and soft tissue infection.
Utility of the QuantiFERON-TB Gold In-tube test (QFT) compared with the Tuberculin Skin Test (TST) in diagnosing tuberculosis in Indian children with malnutrition: A prospective study

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Background: Interferon-gamma release assays like the QuantiFERON-TB Gold In-tube test(QFT) that measure immune response to \textit{M.tuberculosis} antigens have advantages over the traditional tuberculin skin test(TST) that gives false-positive results in BCG-vaccinated children due to common antigens in BCG and tuberculin, and false-negative results in malnourished children due to anergy. This prospective study was done to compare the efficacy of the QFT versus the TST in diagnosing tuberculosis(TB) in children with a high incidence of malnutrition.

Methods & Materials: All children aged 1 to 15 years presenting to the Department of Pediatrics, Christian Medical College, Vellore from February 2010 to April 2011 with suspected TB had TST and QFT tests and AFB cultures done, and other tests as indicated. Study children were classified into one of three WHO-defined groups, namely confirmed TB, clinically diagnosed TB or not TB.

Results: 88 children with suspected tuberculosis were recruited into the study. 88/89 (99%) were BCG-vaccinated. 66.2% of study children were malnourished, with one-third having weights less than 70% expected for age. 24 children had WHO-defined tuberculosis, including 7 confirmed TB. TST was positive \( \geq 10 \) mm in 17 children while QFT was positive in 21 children with 6 being indeterminate. TST and QFT concordance in 80 evaluable children was 79\% overall (kappa 0.430), 57\% in those with culture-confirmed TB, 64\% in those with clinical TB and 85\% in those without TB. All TST+/QFT- discordant results were seen in children without TB. Among 8 children with QFT+/TST- discordant results, 4 had WHO-defined TB including 3 culture-confirmed. Sensitivity of QFT vs. TST was 69.6\% vs 52.9\% for WHO-defined (confirmed and clinical) TB, with specificity 86\% vs 78.3\%, positive predictive value 67\% vs 38\%, and negative predictive value 88\% vs 87\% respectively. These differences between tests were not statistically significant. There was a significant association of negative TSTs with malnutrition less than 70\% of expected weight for age (\( p=0.05 \)) that was not seen with negative QFTs (\( p=0.48 \)).

Conclusion: The QFT performed better than the TST in BCG-vaccinated children. Its significantly better performance in malnourished children supports its use for TB diagnosis in this subpopulation.
Etiology of acute respiratory infections in infants: A prospective birth cohort study

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**Background:** Acute respiratory infections (ARI) is continue to be the leading cause of mortality and morbidity in under five children from infectious diseases worldwide. There are paucity of studies on etiology of ARI in infants from developing countries. Our aims of this study are to document incidence and etiology of ARI in infants, their seasonal variability and association of clinical profile with the etiology.

**Methods & Materials:** A cohort of newborns (310) were followed for the first year of life; for each episode of ARI, nasopharyngeal aspirates were collected and tested for virus with multiplex real time PCR assay. For lower respiratory infections (LRTI) additionally blood culture, serum procalcitonin, serum antibodies to *Mycoplasma* and *Chlamydia* and urinary *Streptococcal pneumoniae* antigen were also assayed.

**Results:** A total of 503 ARI episodes were documented at incidence rate of 1.8 episodes per infant per year. Of these samples were processed in 395 episodes (URTI: 377 & LRTI: 18). One or more viruses were detected in 250 (63.3%) episodes and viral coinfections in 72 (18.2%). Rhino virus (RV) was the commonest virus [105 (42%)] followed by Respiratory syncytial virus (RSV) [50 (20%)], Parainfluenza [42 (16.8%)] and Corona virus [44 (17.6%)]. In LRTIs viruses were detected in 12 (66.7%), bacterial infections in 17 (94.4%) and mixed bacterial–viral infection in 8 (44.4%) episodes. Maximum incidences of most viruses were during month of February- March and September- November. There was no significant difference in symptom duration with virus types.

**Conclusion:** In this cohort of infants, ARI incidence was 1.8 episodes per year per infant; 95% were URTIs. Viruses were identified in 63.3% episodes and the common viruses detected were RV, RSV and parainfluenza virus.
Congenital Syphilis: Complicating an already complex adoption process

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**Background:** With the number of adoptions on the rise both nationally and internationally, screening and subsequent treatment of congenital infections is of particular importance. Among the congenital infections in adoptees, detection of syphilis is particularly difficult, as most affected babies are asymptomatic, especially during infancy. We present the story of an apparently asymptomatic orphan whose VDRL returned positive on a routine adoption screen.

**Methods & Materials:** An 11 month old girl was brought to the pediatric outpatient following VDRL positivity. Maternal and birth details were not known. Following take-over by a non-governmental organization at an approximate age of 5 months, she did not have any history of failure to gain weight, repeated infections, skin lesions, jaundice or bleeding manifestations. On evaluation, she was found to be thriving well and developmentally appropriate for age. General physical examination revealed frontal bossing. There was no lymphadenopathy, rash, rhinitis, condyloma lata or mucocutaneous involvement. Abdominal examination revealed hepatosplenomegaly. Other systemic examination was normal.

**Results:** Following a non-treponemal VDRL test seropositivity, a treponemal test- microhemagglutination test for Treponema pallidum (MHA-TP) was also positive. Blood counts were suggestive of iron deficiency anemia, and there was no thrombocytopenia. Urine examination was normal. Serological tests for HIV were negative. CSF analysis yielded normal sugar, protein and chloride levels. No bacteria were seen on dark field microscopy, cultures were sterile and CSF VDRL test was negative. Radiological evaluation revealed hot-cross bun appearance of the skull with destructive changes, and dental abnormalities consistent with early Hutchinson's teeth. Distortion product oto-acoustic emissions were absent bilaterally, and were suggestive of moderate hearing loss. There was no evidence of interstitial keratitis, chorioretinitis or glaucoma on ophthalmological evaluation. She was treated with Crystalline Penicillin 50,000 units/kg body weight intravenously 6th hourly for 14 days. She has been adopted by a loving family, and is otherwise asymptomatic on follow-up.

**Conclusion:** Although uncommon, congenital syphilis can complicate an already complex adoption process. Infected infants are generally not detected during this period since the disease is most commonly asymptomatic during infancy. This case reiterates the importance of early screening and timely initiation of appropriate treatment which could enhance outcomes in potential adoptees.
Bacteraemia in paediatric: Epidemiology and aetiology at tertiary care centre, Malaysia
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**Background:** Reports of the aetiology of bacteraemia in children from Malaysia are scarce, and have been perplexed by lacking of capacity to identify invasive disease with suboptimal laboratory culture methods. This study aimed to determine aetiological agents causing bacteraemia and its clinical manifestation in paediatric population with their resistance rate towards commonly used antibiotics.

**Methods & Materials:** A retrospective study was conducted by analysing the clinical information details, blood culture and antimicrobial susceptibility testing results using Hospital Information System and statistical software, IBM SPSS version 22, in children between the ages of 0 to 13 years who were admitted to Hospital Selayang, from January 2001 to December 2011.

**Results:** The mean age of the patients was 25.8 months, with infant and children between 1 month and less than 2 years comprised the largest numbers (39%) of the population studied. There were 222 positive blood cultures, with 30 different organisms detected. Gram-positive and Gram-negative bacteria accounted for 46.4% and 53.6% of isolates respectively. Three most commonly isolated aetiological agents were non thypoidal *Salmonella* (n = 38; 17.1%), *Staphylococcus aureus* (n = 38; 17.1%) and *Streptococcus pneumoniae* (n = 28; 12.6%). Non thypoidal *Salmonella* (NTS) isolates demonstrated 18.4%, 10.5% and 2.63% of resistance towards ampicillin, trimethoprim-sulfamethoxazole and ciprofloxacin respectively. All NTS isolates were susceptible to ceftriaxone. *Staphylococcus aureus* isolates exhibited 7.9% of resistance to oxacillin/cefoxitin disc (MRSA), while remaining isolates were susceptible. *Streptococcus pneumoniae* isolates showed 33% of resistance to penicillin. Other important isolated bacteria were *Escherichia coli* (n = 14; 6.3%), and none of the isolates was an extended spectrum beta lactamase (ESBL) producer, in contrast to 10% in *Klebsiella pneumoniae* isolates (n = 10; 4.5%). All *Pseudomonas aeruginosa* isolates (n = 6; 2.7%) were susceptible to ceftazidime. Acute gastroenteritis (80.0%) was the main presentation of NTS bacteraemia, while skin and soft tissue infections with lower respiratory tract infections (31.6%) were common in bacteraemia caused by *Staphylococcus aureus*. *Streptococcus pneumoniae* bacteraemia presented mainly as pneumonia (60.8%).

**Conclusion:** Hence, NTS was the most common aetiological agent isolated in bacteraemia and mainly manifested as acute gastroenteritis.
Five-year review of non-typhoidal salmonella meningitis in Cape Town, 2010 - 2015


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Background: Salmonella species are gram negative bacilli with over 2600 serotypes and a worldwide distribution. Non-typhoidal Salmonella (NTS) typically causes self-limiting gastroenteritis, but may become invasive. In sub-Saharan Africa invasive NTS disease is associated with a high mortality, associated with malnutrition, malaria and human immunodeficiency virus (HIV) co-infection. Meningitis due to NTS is a rare complication, with mortality rates over 40% in children. We report three cases of NTS meningitis in paediatric patients in Cape Town (Note: two more cases have been identified).

Methods & Materials: A five-year review of NTS cultured from cerebrospinal fluid (CSF) at the Division of Medical Microbiology, Groote Schuur Hospital was performed. This is a tertiary academic microbiology laboratory. The National Health Laboratory Service (NHLS) laboratory information system was searched from 1 July 2010 to 30 June 2015. Retrospective clinical reviews were conducted for these cases including patient history, clinical features, risk factors, treatment and outcomes.

Results: From all CSF cultures sent to GSH Microbiology laboratory (n=41865), three cases of NTS meningitis were identified. All three Salmonella meningitis cases were in infants less than one year old. One infant was Zimbabwean with a travel history. All cases were HIV-uninfected: one child was HIV exposed. NTS was isolated from both CSF and blood culture in 2 cases, no blood culture was done on the third. Salmonella enterica serotype Enteritidis (Salmonella Enteritidis) was cultured from two cases and Salmonella Heidelberg from the third. All isolates were susceptible to all antimicrobials tested. One patient died within 72 hours of admission; the remaining two developed neurological complications, including hydrocephalus, hemiplegia and cerebral infarcts.

Conclusion: NTS meningitis should be considered in infants if gram negative bacilli are observed in CSF. The prevalence of NTS meningitis in South Africa appears to be low. A third-generation cephalosporin (Ceftriaxone/Cefotaxime) remains the empiric treatment for meningitis. The duration of treatment for gram negative meningitis is usually 21 days. For NTS meningitis at least 4 weeks of therapy may be indicated to prevent relapses. In view of the poor prognosis and high risk of relapse the use of combination therapy with a cephalosporin and fluoroquinolone, which has enhanced intracellular activity, may be required.
Increased isolation of Enterococcus faecium from neonates with sepsis: An attempt to investigate the suspected outbreak

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Background: Neonatal sepsis accounts for 30-50% of total neonatal deaths in developing countries and there are several reported nosocomial outbreaks of neonatal sepsis. We have noted an increase in the isolation of Enterococcus faecium from blood of neonates admitted in intensive care unit with sepsis. In the present study we report the clinical, microbiology profile and an attempt to investigate the suspected outbreak by E. faecium.

Methods & Materials: Neonates admitted in intensive care units with sepsis in the month of March 2009 were included. Blood cultures collected in Trypticase soy broth and sent to the laboratory were processed conventionally. Identification and antibiotic susceptibility of the isolates were performed by conventional methods and Vitek-2. Case sheets were reviewed for clinical features. E. faecium isolates were subjected to 16S rRNA gene sequencing with fluorescence-labeled dideoxynucleotide terminators using an ABI 3130 XL automated sequencer. The sequences were analysed and identified using the Megablast search program of the GenBank database. The relatedness of sequences of the isolates was studied for any outbreak.

Results: Blood cultures from 40 neonates received during the study period. Blood cultures from 18/40 neonates showed bacterial growth. Nine of 18 (50%) neonates showed the growth of E. faecium. All the E. faecium isolates were susceptible to Vancomycin and Linezolid. Susceptibility to other antibiotics: uniformly susceptible to Quinupristin/Dalfopristin and Chloramphenicol. Resistant to macrolides, fluoroquinolones, Gentamicin(high-level). Sequences of seven of the nine isolates were deposited in GenBank (GenBank accession numbers HM222631 to HM222637). The sequence of each isolate was different from the other. The neonates were either preterm or low birth weight. Babies presented with respiratory distress (6/9), with seizures (2/9) and refusal to feed (1/9).

Conclusion: Among the neonates from whom Enterococcus faecium was isolated in blood, no specific clinical feature could be noticed. The isolates were found to be different from each other in our attempt to establish the relatedness of the strains.
Vaginal colonization by microbes during early pregnancy and their association with adverse pregnancy outcomes

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Background: Role of maternal intrauterine infections/inflammation (IUI) in the causation of adverse pregnancy outcomes (APOs) such as preterm birth (PTB), low birth weight (LBW) and preterm premature rupture of membranes (pPROM) is well acknowledged. In many instances, ascent of colonizing microbes from lower genital tract (LGT) is reported to cause IUIs and the associated APOs. Despite the high occurrence of PTB and LBW among developing nations such as India, there is a severe scant of knowledge regarding the colonization rates of various microbes in the LGT during pregnancy and more importantly their influence on the pregnancy outcomes.

Methods & Materials: A hospital based cohort study was undertaken comprising of 790 pregnant women in the age of 18-35 years and gestational age of 8-24 weeks at a secondary care hospital in south India. Upon recruitment, high- vaginal (HV) and endocervical swabs (EC) from all study participants were collected. All HV swabs were subjected to microbiological culture techniques. PCR based detection of genital mycoplasmas and C. trachomatis DNA was performed from HV and EC swabs respectively. Data was analysed using SPSS software. Detection rates of individual bacteria and in combinations was analysed using descriptive statistic tools and 2x2 tables. Association of microbes with APOs was estimated using chi square test and univariate analysis.

Results: Majority (43%) of the study participants were within 12-16 weeks of gestation at the time of sampling. Vaginal colonization of Candida spp (15.7%) followed by anaerobic GNB (98, 12.4%) and Trichomonas vaginalis (94, 11.9%) were predominantly observed among the study participants. Presence of any one of the genital mycoplasmas was observed among 9% of the women. None of the study subjects were positive for C. trachomatis. PTB, LBW and pPROM was observed among 7.6, 11.4 and 1.4% women respectively. Colonization of G. vaginalis, anaerobic GNB and M. hominis were significant risk factors for PTB, while anaerobic GNB and M. hominis were for LBW and presence of T. vaginalis and U. urealyticum were significant risk factors for pPROM in the study population.

Conclusion: Our study findings underscore the need for routine microbiological screening of pregnant women for LGTIs and vaginal dysbiosis during early pregnancy.
Is the QuantiFERON-TB Gold test (QFT) better than the Tuberculin Skin Test (TST) in diagnosing active and latent tuberculosis in BCG-vaccinated children?

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Background: Interferon-gamma release assays like the QuantiFERON-TB Gold test (QFT) have advantages in diagnosing tuberculosis (TB) over the Tuberculin Skin Test (TST) that gives false-positive results in BCG-vaccinated children due to common antigens in BCG and tuberculin. This study was done to compare the QFT and TST in the diagnosis of active and latent TB in predominantly BCG-vaccinated children.

Methods & Materials: Retrospective cohort study of children aged 1 to 15 years evaluated for tuberculosis with TST and QFT testing in the Department of Pediatrics, Christian Medical College, Vellore from January 2007 to December 2010. The Department of Clinical Microbiology’s QFT database was accessed and records of study-eligible children reviewed for demographic data, TST results and final diagnosis.

Results: 175 children underwent both TST and QFT testing, of whom 173 (99%) were BCG-vaccinated. 30 children were diagnosed with tuberculosis based on WHO criteria. TST was positive ≥10 mm in 43 while QFT was positive in 27 children. TST and QFT concordance in 168 evaluable children was 83.2% in culture-confirmed TB and 87.1% in those with negative AFB cultures; 78% in 27 children with clinical TB (kappa 0.526) and 85% in 141 without tuberculosis. Sensitivity of TST and QFT in culture-confirmed TB was 66.7% and 71% respectively, with specificity of 84.2% and 79% and negative predictive value of 97.8% for both. Sensitivity of TST and QFT in clinical TB was 48.8% and 55.6% respectively with specificity of 93.2% and 91.5%. The QFT had 58.3% sensitivity and 95.3% specificity in diagnosing children with latent TB. The commonest discordant results were TST+ / QFT- in 15 of 141 children without TB, not unexpected in this BCG-vaccinated population.

Conclusion: The QFT performed better than the TST in the diagnosis of tuberculosis. Although only moderately sensitive, they were highly specific in ruling out TB and showed good concordance in TB-negative children. Although a case may be made for using both tests in BCG-vaccinated children, the higher costs and technical expertise required for the QFT do not support its use instead of the cheaper and simpler TST in India.
Accuracy of the Xpert MTB/RIF assay compared to the “gold standard” AFB culture in the diagnosis of tuberculosis in children in India.

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Background: The Xpert MTB/RIF PCR assay detects *M. tuberculosis* and resistance to Rifampicin within 2-4 hours, compared to the AFB culture that takes 4-8 weeks to provide the same information. This study assessed the performance of the Xpert MTB/RIF assay against the AFB culture and clinical case definitions of intrathoracic and extrathoracic tuberculosis (TB), and against type of specimen tested.

Methods & Materials: All children < 16 years of age with suspected TB seen in the Pediatric Department, Christian Medical College, Vellore from May 2012-June 2015 and tested with Xpert MTB/RIF assay and AFB cultures in the Department of Clinical Microbiology were included. Records and test results were accessed to classify children as confirmed, probable or possible TB or TB unlikely/not TB based on the 2014 NIH/WHO consensus and other case definitions.

Results: 286 children with suspected TB (135 intrathoracic, 71 lymphadenitis, 51 meningitis and 28 abdominal) were reviewed. 41 of 52 children with culture-confirmed TB had a positive Xpert MTB/RIF assay (overall sensitivity 78.9%). 115 more children were case-defined TB, while 119 had no TB. The sensitivity of the Xpert MTB/RIF assay against the AFB culture was 96% in intrathoracic TB, 78% for TB lymphadenitis, 67% for TB abdomen and 46% for TB meningitis. The assay showed less sensitivity against clinical case definitions, being 48% for intrathoracic TB, 47% for TB lymphadenitis, 41% for TB abdomen and 19% for TB meningitis. The assay diagnosed an additional 15 children with intrathoracic TB, 11 children with TB lymphadenitis, and 3 and 2 children with TB abdomen and TB meningitis compared to the AFB culture. Sensitivity was 100% for sputum specimens, intrathoracic tissue and BAL samples, 90% for gastric aspirates, 78% for lymph nodes, 67% for intra-abdominal tissue and 46% for CSF against the AFB culture. It was 80% sensitive in detecting Rifampicin resistance. The specificity of the assay in disease-negative children was 92% for intra-thoracic and abdominal TB, 93% for TB lymphadenitis, and 100% for TB meningitis.

Conclusion: The Xpert MTB/RIF assay’s high sensitivity, specificity and detection of Rifampicin resistance make it a good point-of-care test for early diagnosis of childhood tuberculosis, especially intrathoracic TB.
Population structure and molecular epidemiology of human clinical multi-drug resistant (MDR) Escherichia coli strains from Pune, India

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**Background:** Extraintestinal pathogenic *E. coli* (ExPEC) cause different infectious diseases in humans and animals, accounting 80% of urinary tract infections (UTIs), worldwide. Increase in the prevalence of multidrug-resistant (MDR) *E. coli* and global dissemination of these clonal organisms is a significant risk to the public health. The present study investigates the prevalence, population structure, phylogenetic affinities and molecular basis of ESBL and antimicrobial resistance and also highlights the spread of multi-drug resistant *E. coli* causing infections with varied clinical spectrum

**Methods & Materials:** A total of 187 *E. coli* isolates from patients with different clinical complications were obtained from D.Y. Patil Medical College, Pune, for this study. All the isolates were subjected to phylogenetic grouping and specific sequence type (ST) identification. The ST131 strains were further evaluated for their virulence gene profiles using PCR. Phenotypic and genotypic detections of antimicrobial resistance were carried out by disc diffusion and PCR based methods, respectively. The clonality of ST131 isolates with respect to strains of other sequence types was evaluated by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR).

**Results:** Phylogrouping of 187 *E. coli* isolates revealed that 78 (41.7%) isolates belonged to group A, 16 (8.6%) to group B1, 56 (29.9%) to group B2 and 37 (19.8%) to group D. Forty strains were identified to belong to ST131. Virulence profiling of ST131 strains demonstrated significant prevalence of *fimH* (90%), *papC* (82.5%), *hlyA* (77.5%) *traT* (47.5%), *sat* (87.5%) and *iucD* (55%). The antibiotic resistance profiles of ST131 isolates showed high resistance to ciproflaxin (92.5%), cotrimoxazole (67.5%) and tetracycline (62.5%). A high proportion (60%) of these ST131 strains was multidrug resistant and 95% were ESBL-positive. Of all the 40 ST131 strains, 42.5% and 95% were positive for sulphonamide and streptomycin resistance encoding genes, respectively, and only 2 strains were found to be positive for ndm-1.

**Conclusion:** Our study revealed that ST131 is the only predominant endemic clone present in the clinical *E. coli* isolates from Pune. Molecular characterization of these isolates suggests that ST131 is a robust lineage of pathogen that could significantly limit medical interventions against *E. coli* induced enteric and extraintestinal infections in India.
Antibiotic resistance among gastrointestinal and respiratory tract bacterial pathogens in Mauritius

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Background: Antibiotic resistance rates vary markedly according to geographical region. It is thus essential to have local data on antibiotic resistance to determine the choice of antibiotics for empirical treatment of bacterial infections. A survey was therefore conducted to determine antibiotic resistance rates in 2014 of the main bacterial causes of gastroenteritis and respiratory tract infections in humans, namely nontyphoidal Salmonella, Campylobacter, Streptococcus pneumoniae and Haemophilus influenzae.

Methods & Materials: Laboratory registers at the two government laboratories which perform microbiological examination were reviewed. Antibiotic susceptibility results of all nontyphoidal Salmonella and Campylobacter from stool specimens, and pneumococcus and H.influenzae from sputum, sinus and ear specimens were recorded. Duplicate isolates from the same patient were excluded.

Results: In 2014, 115 Salmonella isolates were recorded and susceptibility rates to ampicillin, co-trimoxazole, nalidixic acid and ciprofloxacin were 100%, 98%, 99% and 100% respectively. In contrast only 49% of 101 Campylobacter isolates were susceptible to ciprofloxacin although 96% were susceptible to erythromycin. Among respiratory tract pathogens, only 21 of 48 (44%) of pneumococcus isolates were susceptible to erythromycin and 63% had reduced sensitivity to penicillin (Minimum inhibitory concentration [MIC] ≥0.1 µg/mL) but none had high level penicillin resistance (MIC >2 µg/mL). 96% and 100% of pneumococci were susceptible to tetracycline and levofloxacin respectively. 27 of 35 (77%) of Haemophilus influenzae isolates were susceptible to ampicillin and all were susceptible to cefotaxime, amoxicillin-clavulanic acid combination and levofloxacin.

Conclusion: High rates of resistance were observed for some organism-antibiotic combinations in Mauritius in 2014 such as for ciprofloxacin in Campylobacter and erythromycin in pneumococcus. When compared to historical data, resistance to ciprofloxacin in Campylobacter in Mauritius increased from 3.6% in 1998-2005 to 51% in 2014 and this was probably due to antibiotic overuse in veterinary practice. In severe bacterial gastroenteritis requiring antibiotic therapy, ciprofloxacin is likely to be effective against Salmonella but not against Campylobacter. Macrolide antibiotics cannot be relied upon as monotherapy to treat infections likely to be caused by pneumococcus in Mauritius. Control of antibiotic use is required in both medical and veterinary practice to minimize the emergence and spread of antibiotic-resistant bacteria.
Re-emergence of susceptibility to conventional first line drugs in Salmonella isolates: an old weapon to fight NARS

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Background: Enteric fever, caused by Salmonella is a common clinical diagnosis among febrile patients presenting to hospital in Nepal. The first-line drugs ampicillin, chloramphenicol and cotrimoxazole have not been part of empirical therapy due to development of multidrug-resistant Salmonella. Ciprofloxacin has been the empirical therapy of choice, but the recent increase in minimum inhibitory concentration (MIC) to ciprofloxacin in Salmonella enterica, may result in delayed response and serious complications. The current study investigates the re-emergence of sensitivity to conventionally used drugs among strains of S. Typhi and S. Paratyphi A in a community hospital of Kathmandu.

Methods & Materials: We evaluated 245 Salmonella isolates, at Helping-Hands Community Hospital, Chabahil, Kathmandu, for chloramphenicol, ampicillin and cotrimoxazole susceptibility using standard methods as per the guidelines of the Clinical and Laboratory Standards Institute.

Results: Of the total 245 positive isolates, 50.20% (123/245) were S. Typhi and 49.80% (122/245) were S. Paratyphi A. A total of 229 (93.5%) were Nalidixic Acid Resistant (NAR) Salmonella, including 110(89.4%) S. Typhi and 119(97.5%) S. Paratyphi A. More than 95% of the isolates were sensitive to ampicillin, chloramphenicol, cotrimoxazole, ceftriaxone, and cefixime.

Conclusion: First generation drug were found effective indicating re-emergence of susceptibility to conventional first line antimicrobial which may play important role in the treatment of NAR and non MDR Salmonella isolate.
Non-typhoidal Salmonella urinary tract infection: Molecular resistance and clinical correlation – A four year study from a tertiary care centre


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Background: Extra intestinal manifestations due to Non-typhoidal Salmonella (NTS), especially bacteriuria are rare. The aim of the study was to determine the incidence, clinical spectrum of disease, phenotypic and genotypic resistance patterns among the isolates and clinical outcomes.

Methods & Materials: Salmonella spp isolated from urine specimens at our centre were studied retrospectively between January 2011 and December 2014. All the isolates were identified biochemically, serotyped according to Kauffman and White antigenic scheme and confirmed at the National Salmonella & Escherichia center, Central Research Institute, Kasauli, India.

Antibiotic susceptibility testing was performed according to CLSI guidelines. Further, molecular characterization was performed to detect resistant genes dhfr1, Sul 2, bla-OXA, bla-TEM, Amp, CTX-M-1, qnr (A,B,S), class 1 and class 2 for sulphonamide, beta-lactam, quinolone and integron resistance respectively.

Clinical outcomes were determined through a review of medical records.

Results: Over four years 1,86,298 urine specimens were received. Salmonella spp was isolated in 36 specimens among which NTS was identified in 23 specimens. Concomitant blood cultures were positive in 27% of cases. Salmonella group C1 (n=9) followed by Salmonella group C2 (n=5) were the most common species isolated. The other groups identified were Salmonella Typhimurium (n=4), Salmonella group E (n=3) and Salmonella group D (n=1).

Phenotypic resistance was seen in 50% isolates to nalidixic acid and 30% to ciprofloxacin and 5% to ceftriaxone. 47% of isolates carried resistant genes and the presence of class 1 integrons while 5% class 2 integrons.

Clinically, the predisposing factors included urologic abnormalities (26%) and immunosuppression due to infections, immunosuppressive drugs, diabetes, pregnancy and age related immunosuppression (30%). Most patients were women (65%). At presentation, 43% of patients presented with fever and dysuria. Only one presented with gastroenteritis. None were treated with the drugs toward which resistant genes were detected.

Conclusion: Immunosuppression, urologic abnormalities and the female gender remained as the most common predisposing factor. Salmonella group C1 the most common group and high prevalence of genotypic integron mediated resistance is seen among NTS isolates causing urinary tract infections.
Burden of rotavirus gastroenteritis and distribution of rotavirus strains in India: A systematic review and meta-analysis
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Background: Rotavirus is the leading cause of severe diarrhea and diarrhea associated morbidity in Indian children. Vaccination is one of the most effective approaches to reduce the morbidity. Vaccine composition depends on the prevalence of most common strains. Therefore, it is important to understand the burden of rotavirus gastroenteritis (RVGE) and prevalence of different strains. This systematic review was aimed to estimate the burden of rotavirus gastroenteritis (RVGE) and distribution of rotavirus strains in India.

Methods & Materials: A systemic search of published literature was carried out using PubMed, Elsevier ScienceDirect, Cochrane library databases and Google scholar (from 1990 to April 2015) by two independent reviewers. Reference list of the related articles was also screened to find out the relevant studies. Newcastle Ottawa scale was used to assess the study quality. Publication bias was assessed by using funnel plot. Cochrane Q-statistics test and I² statistics were used to assess the heterogeneity. Random effect model was used. Comprehensive Meta-Analysis software (Version 2.2, Biostat, Englewood NJ) was used.

Results: A total of 44 studies, covering a total of 33313 hospitalized children suffering from diarrhea. Maximum number of studies belongs to north followed by studies from south, east and west part of country (15, 10, 7 and 5 studies, respectively). Seven studies were Multi-centric. The number of participants varied from 106 to 6765. The proportion of gastroenteritis cases due to rotavirus was 23.9% (95% confidence interval [CI], 21.1-26.9). Among G type, G1 was found most commonly prevalent followed by G2 and G9 (27.7%, 25.8% and 13%, respectively). The most common P type were P8, P4 and P6 (31.9%, 24.9%, and 11.1%, respectively). G2P[4] was found most prevalent in GP combinations, followed by G1P8 and G9P8 (16.5%, 16.1%, and 6.0%, respectively). The most prevalent GP combinations were G3P[8] (32.1%), G1P[8] (23.0%), and G2P[4] (7.9%).

Conclusion: Rotavirus gastroenteritis (RVGE) is an important cause of diarrheal disease in Indian children. G1 and P8 were found most prevalent strains in G and P type, respectively. Vaccination programme with broad serotype vaccine may help in decreasing the RVGE burden in India.
Development of predictive dengue risk map using Random Forest

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**Background:** Dengue fever, a disease caused by Dengue virus (DENV) and transmitted to humans by *Aedes aegypti* and *Aedes albopictus* mosquitoes, has been hyper-endemic in Singapore for several decades. In the absence of an effective vaccine or specific treatment to mitigate the infections, control of *Aedes* mosquitoes plays a critical role in controlling the disease. In recent years, Singapore’s vector control operations have been overwhelmed by geographical expansion of dengue transmission as well as increasing magnitude of epidemics. At the same time, they have been hampered by the lack of tools to assess the impending risk of dengue fever outbreaks spatially and manpower insufficiency.

**Methods & Materials:** To help operation department allocate limited resources, we developed a predictive risk map for dengue transmission using the Random Forest algorithm, incorporating various risk factors and accounting for temporal and spatial lag effects of the factors. A wide range of factors representing the characteristics of past dengue situation (total number of cases in previous year and number of non-resident cases in previous year), human population (estimated population density), vector population (estimated ratio of *Aedes aegypti* mosquitoes out of all *Aedes* mosquitoes—breeding percentage) and environment (vegetation index, connectivity index and ratio of residential area) were examined and incorporated in the model.

**Results:** Validation using most recent data showed that the observed and the predicted risk ranks had a Pearson correlation of 0.87 (P<0.001) and a weighted Kappa agreement of 0.814 (P<0.001) when categorised to risk groups. In addition, the model was able to estimate the partial effects and relative importance of individual risk factors, which can strengthen our understanding of the risk factors of dengue transmission.

**Conclusion:** Our risk map has strong predictive capability, hence may be an important tool in guiding targeted vector control interventions for dengue.
Hand hygiene program: "Go for 100**". Whole impact (hospital cost, MRSA attack, nosocomial infections and device related infections)

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Background: Nosocomial infections (NI) are significant cause of mortality and hospital costs. Annual costs have approximately estimated of US$6.8 billion in the USA. Hand hygiene is the most important measure to prevent NI reducing them a 30% on average and to reduce MRSA attack rate. There is spare information about this in middle income countries. The aim was to explore the impact of the program in hospital costs, nosocomial infections and MRSA attack rate and clonality.

Methods & Materials: Program "Vamos por el CIEN -Go for 100" (CIEN Spanish acronym of infection control by integration and innovative strategies): based on the WHO hand hygiene multimodal strategy but focalizing education and awareness for every hospital sector and adding periodically innovative strategies. Here we analyze 2013-2015. Direct costs of this program were evaluated monthly. Active nosocomial infections surveillance was done. A collection of 43 S. aureus clinical isolates from pediatric patients (one isolate per patient) was collected from January 2012 to May 2015. Molecular genotyping assays. Pulsed-field gel electrophoresis (PFGE) in the S. aureus clinical isolates was performed. The DNA fragment patterns generated by PFGE were analyzed using NTsys program 2.0 with the Sorencen-Dice coefficient and the unweighted pair group method with an arithmetic mean (UPGMA) clustering system.

Results: Over 1,000 sessions took place from 2013-2015, impacting more than 15,000 people, the consumption of BPA increased from 125L/month to 400L/month, 2400 posters were allocated, the cost of the program per year was $217,618 dlls. Hand hygiene compliance increased from 48% to 74%, NI decreased from 7.68 to 6.47/100 discharges (15.7%), and the MRSA attack from 8.3 to 2.13/100 discharges. High clonality among nosocomial isolates MRSA was found, suggesting the presence of clones scattered in hospital environment that can interfere with program strategies.

Conclusion: Probably due to a better distribution of resources the program costs were lower than the year before the program ($3743031.7 dlls). We also demonstrate that in a middle income country a hand hygiene program can decrease in NI especially in S. aureus. Phylogenetic analysis showed that hand hygiene has a direct impact on the clonality of MRSA hospital origin.
Morbidity, mortality, and seasonality of influenza hospitalizations in Egypt, November 2007 - November 2014
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Background: Influenza typically comprises a substantial portion of acute respiratory infections, a leading cause of mortality worldwide. However, influenza epidemiology data are lacking in Egypt. We describe seven years of Egypt's influenza hospitalizations from a multi-site influenza surveillance system.

Methods & Materials: Syndromic case definitions identified individuals with severe acute respiratory infection (SARI) admitted to eight hospitals in Egypt. Standardized demographic and clinical data were collected. Nasopharyngeal and oropharyngeal swabs were tested for influenza using real-time reverse transcription polymerase chain reaction and typed as influenza A or B, and influenza A specimens subtyped.

Results: From November 2007–November 2014, 2,936/17,441 (17%) SARI cases were influenza-positive. Influenza-positive patients were more likely to be older, female, pregnant, and have chronic condition(s) (all p<0.05). Among them, 53 (2%) died, and death was associated with older age, five or more days from symptom onset to hospitalization, chronic condition(s), and influenza A (all p<0.05). An annual seasonal influenza pattern occurred from July–June. Each season, the proportion of the season's influenza-positive cases peaked during November–May (19–41%).

Conclusion: In Egypt, influenza hospitalizations cause considerable morbidity and mortality and its seasonality mirrors Northern Hemisphere patterns. Additional assessment of influenza epidemiology in Egypt may better guide disease control activities and vaccine policy.
Fecal microbiome therapy in relapsing Clostridium difficile infection – long-term results
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Background: Fecal microbiome therapy (FMT) has become an accepted rescue treatment for relapsing or recurring Clostridium difficile infection (CDI). While short-term effectiveness of this treatment approach is high, no data on the long-term efficacy are available.

Methods & Materials: From 17 patients (median age 84, range 55-93 years) who underwent 18 FMT procedures for relapsing or recurring CDI long-term outcomes were analysed. FMT was done via endoscopically placed jejunal tube using 500 ml (0.1 g/ml) of sterile prepared donor stool suspension from healthy first-degree relatives (n=15) or spouses (n=3).

Results: All patients had a Charlson comorbidity score > 10 indicating multiple comorbidities. Median number of treatment courses for CDI before FMT was four (range 3-7). Postprocedure efficacy for FMT was 17/18 (94.4%). At day 30 14/18 (77.8%) were without clinical symptoms or signs of relapse, by day 180 7/10 (70%) were free from CDI, and after one year 6/9 (66.7%). Leukocyte counts, albumin, clinical response measured by the use of the Bristol Stool Chart (BSC) or stool lactoferrin at baseline were not predictive for long-term response whereas a >75% decrease after FMT in stool lactoferrin concentrations by day 7 compared to day 0 was indicative.

Conclusion: Even in elderly patients with severe or multiple comorbidities and high risk of recurring CDI the use of FMT protects a substantial number of patients over more than one year. The decrease in stool lactoferrin concentrations within one week after FMT remained the only predictive biomarker for long-term response in these patients.
Plasmodium falciparum malaria: association of sickle cell trait in the reduction of parasite density in symptomatic Fulani tribe living in sympatry in Mali, West Africa

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**Background:** Mutation of the b-chain of the globin gene due to a single base pair mutation A→T in the genome sequence has been associated with protection from severe malaria outcome. Differences in malaria susceptibility have been accorded to asymptomatic Fulani and other sympatric ethnic groups in Burkina Faso and Mali with Fulani being less parasitized, infected and more responsive to *Plasmodium falciparum* antigens.

**Methods & Materials:** In this study, we have examined symptomatic individuals of different ethnicity (123 Fulani and 254 Dogon) in Mali, genotyped for haemoglobin S and also assessed their antibody levels to crude asexual blood-stage antigen.

**Results:** We found that Fulani individuals with HbAA had a statistically significantly higher parasite density when compared with their HbAS counterparts [OR 1.9 (95% CI, 1.6-2.3, P<0.001)]. The results also showed that parasite density in Dogon tribe were statistically higher than that of the Fulani tribe, irrespective of their haemoglobin status. We also found a significantly inverse correlation with parasite density and age of HbAS Fulani individuals ($R^2$=0.549, P=0.0018). No correlation between anti-malarial antibodies and haemoglobin AA or AS was observed.

**Conclusion:** Sickle cell trait could be another contributing factor to the immuno-genetic differences observed in Fulani living in sympatry with other ethnic groups in West Africa.
Diagnosis of Mycoplasma pneumoniae infection in children by using serology and polymerase chain reaction in community-acquired lower respiratory tract infections

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Background: *Mycoplasma pneumoniae* plays a significant role in lower respiratory tract infections (LRTIs) in children. The aim of this study was to investigate the role of *M. pneumoniae* in community-acquired LRTIs in children, with use of both serological tests and PCR analysis.

Methods & Materials: A total of 100 patients 6 months to 12 years of age with acute lower respiratory tract infections were enrolled for this study. These patients were investigated clinically and radiologically. Sera were used for detecting IgM and IgG antibodies to *M. pneumoniae* by ELISA. PCR to amplify fragments in the 16srDNA gene of *M. pneumoniae* was conducted employing throat swab samples.

Results: In the present study, there were 61 (61%) male and 39 (39%) female children had LRTIs but no statistical significant association was found between sex of the patient and incidence of *M. pneumoniae* infection. Eighteen (60%) patients were positive for *M. pneumoniae* in the age group of ≤5 years and 12 (40%) patients of ≥5 years of age were positive for *M. pneumoniae* but this difference was found to be statistically significant. (p=0.02). The clinical and radiological profile across *M. pneumoniae* positive and negative cases were comparable. Serological evidence of acute infection was observed in 28 (28%) patients. PCR was positive in throat swab samples of 6 (6%) patients; 4 (66.6%) patients with serologically proven and 2 (33.3%) serological unproven. Together, serology and/or PCR detected thirty (30%) patients *M. pneumoniae* infection.

Conclusion: In conclusion, our data underline the role of *M. pneumoniae* in children with community-acquired LRTIs, even in children aged <5 years.
Quantifying tuberculosis burden and underrepresentation in Malaysia, 1990-2014
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Background: Tuberculosis continues to be the leading infectious disease threat in Malaysia. With recent spikes of burden following the newly intensified case finding programme introduced since year 2011 we estimated how much the national data reflected the actual burden hence objectively quantified the underrepresentation by using mathematical modelling technique.

Methods & Materials: We reviewed tuberculosis pathophysiology and its transmission dynamic that best reflected reality at present which includes primary infection, endogenous reactivation and exogenous reinfection. We used the Malaysian national tuberculosis data from year 1990 till 2014 and constructed a deterministic compartmental model with SEIR structure and ordinary differential equation system. We took into account its unique characteristics on heterogeneity mainly age and gender. Model fitting, probabilistic sensitivity testing and uncertainty analysis were performed. Retrospective projection of the Malaysian tuberculosis cases estimated between year 1990 till 2014 were produced. The model then was compared with the observed data within similar years and further quantified how many cases were underrepresented.

Results: A steady and higher increasing trend of tuberculosis cases were estimated from year 1990 till 2014 between 14,032 to 22,260 cases with annual incidence rate between 1.0% to 5.5% than the national observed number of cases between 11,702 to 24,711 cases with similar annual incidence rate. Further analysis showed underrepresentation rates ranging between 0.32% to 26.84% from year 1990 till 2014. Comparison between model estimates and the national observed number of cases from year 1990 till 2011 showed an annual mean case underrepresentation of 13.49% (95% CI: 10.40;16.58). A slightly lower annual mean case underrepresentation of 13.11% (95% CI: 10.39;15.84) was estimated from year 1990 till 2014 in line with the newly intensified case finding programme introduced since year 2011 onwards.

Conclusion: We conclude that the current Malaysian observed data has an underrepresentation of tuberculosis cases ranging between 13.11% to 13.49%. This knowledge discovery is imperative to objectively complement current work on disease and economic management programmes for greater impact resulting from higher rates of case detection and treatment hence reducing incidence.
TB outbreak investigation in a faith based boarding school: Challenges and control measures

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Background: Tuberculosis burden in UK is unacceptably high compared to similar countries. TB outbreaks in schools are extremely complex and present public health challenges. 7 cases of TB were notified at a faith based female boarding school in a UK city over 5 years. We aim to describe this challenging outbreak investigation.

Methods & Materials: In 2008, the first pulmonary TB case was notified to public health authorities. In subsequent years, more cases were reported. Epidemiological investigations were undertaken in school including risk assessment, contact tracing and screening following each case to identify cases of active or latent TB. Mass screening at school was extremely challenging both in terms of logistics and managing cultural sensitivities. Initially, TB strain typing testing was not available. In 2013, following a case, the whole school was screened again. In early 2014, retrospective review and 24 loci TB strain typing was done to identify transmission chains.

Results: From 2008 to 2013, mass TB screening was done four times in this school. In total, 1524 students and staff were screened. Of these, 98 had latent and 13 had active TB. In 2012, 64 cases (11%) had latent TB and 9 (1.6%) had active TB. Epidemiological investigations did not reveal any chains of transmission. TB strain typing in 2014 revealed that the 2012 case had identical 24 loci strain typing to the 2008 case. Strain typing was not possible in extra-pulmonary TB cases, and cases with missing loci could not be linked microbiologically.

Conclusion: Strain typing data suggests ongoing TB transmission in school. Finding latent and active TB cases in school does not indicate exposure to notified cases, as most students are from a high risk population group. Whole genome sequencing could provide accurate data on ‘lineages’ of M. tuberculosis for evidence on chains of transmission. This outbreak highlights the need for innovative TB prevention and control strategies in such settings. Control measures included risk assessment and screening of new students, BCG vaccination history, prompt referral of symptomatic cases to TB services and raising awareness about TB. Proactive control measures in such high risk settings can minimise spread and prevent future outbreaks.
Age-dependent carriage of alleles and haplotypes of Plasmodium falciparum sera5, eba-175, and csp in a region of intense malaria transmission in Uganda

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**Background:** The development of malaria vaccines is constrained by genetic polymorphisms exhibited by Plasmodium falciparum antigens. We investigated the age-dependent distribution of alleles or haplotypes of three *P. falciparum* malaria vaccine candidates, circumsporozoite protein (csp), erythrocyte binding antigen 175 (eba-175) and serine repeat antigen 5 (sera5) in a region of intense malaria transmission in Uganda.

**Methods & Materials:** A cross-sectional study was carried out between August and November 2009. Blood samples were collected after informed consent from 250 individuals below 5 years, 5-10 years and above 10 years old. *P. falciparum* DNA was extracted from all samples. Alleles of sera5 and eba-175 were determined by polymerase chain reaction (PCR) amplification followed by resolution of PCR products by agarose gel electrophoresis and allele calling using photographs of ethidium bromide-stained gels. Haplotypes of CSP were identified by sequencing 63 PCR products and using *P. falciparum* 7G8 strain sequence as a reference.

**Results:** Both eba-175 FCR3 (48/178) and CAMP (16/178) alleles were observed with the FCR3 (24/67) allele being predominant among children aged below 5 years old while the CAMP (12/67) allele was predominant among older individuals. Both sera5 alleles ORI (6/204) and ORII (103/204) were observed in the population but ORII was more prevalent. SERA5 ORII allele was significantly associated with age (P values < 0.0001), parasite density (P value < 0.0001) and clinical outcomes (P value= 0.018). There was marked CSP diversity in the Th2/Th3 region. Out of 63 sequences, 16 conformed to the reference strain and one (1/16) was similar with a West African haplotype and the majority (14/16) of the haplotypes were unique to this study region.

**Conclusion:** There was an age-dependent distribution of CSP haplotypes with more haplotypes being harbored by < 5-year olds, (10/16) compared to adults (2/16). Interestingly, the CSP haplotype corresponding to 3D7 whose prototypical sequence is identical to the sequence of the leading malaria vaccine candidate RTS, S was not observed. Our data suggest that eba-175 FCR3 allele, sera5 ORII allele, and CSP haplotypes are targets of host immunity and under immune selection pressure in Apac District.
Occurrence of novel and emergent tick-borne pathogens in a Kenyan biodiversity hotspot

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**Background:** Ticks are important vectors of emerging infectious zoonotic pathogens that significantly contribute to public and animal health burdens. Limited understanding of pathogen diversity and their tick vectors especially in areas of intensified human-livestock-wildlife interactions often constrains development of better disease prevention and control strategies. Here, we aimed to gain insight on diversity of pathogens and their tick vectors collected from Shimba Hills National Reserve (SHNR), one of Kenya’s high biodiversity areas facing considerable human encroachment.

**Methods & Materials:** We collected questing ticks from six sites using CO₂ traps, flagging as well as direct handpicks from vegetation. Morphologically, adults were identified to species level while nymphs and larvae were identified to the genus level under a stereo microscope using taxonomic keys. Molecular analysis of CO1, ITS2 and 16S rRNA genes was used to further confirm adult species identifications and to assign species identities to the nymphs and larva which were difficult to identify based on morphological characteristics. We pooled the ticks in varying sizes, depending on species and life-cycle stages, and screened for arboviruses as well as bacterial and protozoan tick-borne pathogens using PCR with high resolution melting analysis and sequencing of unique melt profiles.

**Results:** We identified three adult tick species (*Amblyomma eburneum*, *Amblyomma thollonii*, and *Rhipicephalus maculatus*) of two genera from 4,324 questing ticks (209 adult ticks, 586 nymphs and 3,502 larvae). Of the 209 adults, 56% were *A. eburneum* while *R. maculatus* and *A. tholonii* were 42% and 2% respectively. From our pathogen screening, we report the first molecular detection of zoonotic *Anaplasma phagocytophilum*, a novel *Rickettsia*-like and *Ehrlichia*-like species in *R. maculatus* ticks. We also detected *Ehrlichia chaffensis*, *Coxiella sp.*, *Rickettsia africae* and *Theileria velifera* in *A. eburneum* ticks for the first time.

**Conclusion:** Our results demonstrate previously unidentified tick-pathogen relationships and a unique tick diversity in the SHNR that may contribute to livestock, and possibly human, morbidity in the region. We emphasize that routine surveillance in similar areas to elucidate disease transmission dynamics and identify novel potential pathogens is critical to guide the development of better tick-borne disease diagnosis, prevention and control measures.
Rising trend of seroprevalence of human amoebiasis in tertiary care hospital of North India

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**Background:** The present study is retrospective analysis of the data of all patients who came for the determination of anti-amoebic antibodies in sera measured by ELISA over the period of 8 years.

**Methods & Materials:** This retrospective study was conducted at our tertiary care hospital in North India. The case records of all patients presenting to the outpatient department or admitted to the wards and intensive care units of the hospital with clinical suspicion of amoebiasis from 2007 to 2014 were reviewed in detail. The serum sample obtained were maintained at -20°C until use. The ELISA was performed for all samples. Qualitative estimation of serum immunoglobulin G (IgG) antibodies to *E. histolytica* was performed using the in-house indirect IgG ELISA from Jan 2007 to June 2013. Later on in-house ELISA was replaced by commercial ELISA (RIDASCREEN® *Entamoeba histolytica* IgG (K1721) kit.

**Results:** A total of 3136 samples from clinically suspected cases were evaluated; overall seropositivity was 61.5% with the predominance of males (85.8%). Most of the patients were adults (92.5%). Inpatient had higher seropositivity rate (55%) when compared to outpatients (45%). No significant seasonal variation was observed in seropositivity rate during this period. The prevalence varied from 47.7% to 78.7% for year 2007 to 2014, depicting an overall raising trend in seropositivity.

**Conclusion:** In conclusion, continuous surging pattern in the disease prevalence was seen over the years which are giving alert to take vigilant action to check the incidence and prevalence of this parasitic disease.
Polyparasitic infections in Coeliac disease – a newer paradigm

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Background: Polyparasitism is an entity which has been increasingly reported in recent times. Literature reports of more than a single organism being involved as a causative agent is more common, however concomitant infections of more than two etiological organisms are relatively rare. Polyparasitism is mostly reported in younger age group as well as in immunocompromised individuals with acute presentations and severe symptoms. Polyparasitism might be driven either by continuous exposure to source of infection of the implicated parasites or possibly by immunological predisposition of the host.

Methods & Materials: Stool microscopy to find out parasitic etiologies was conducted in patients (n=200) with gastrointestinal disorders using direct and concentrated techniques as well as using various staining techniques.

Results: A 19 year male from rural background with coeliac disease, without having any known immunosuppression (Antibodies to HIV, HBV, HCV were negative) presented with complaints of weight loss, chronic loose stools alternating with constipation and pain abdomen since last 7 months. Macroscopic examination of stool was not significant. On microscopic examination, several ova of helminths and cysts of protozoa were detected. Ova of Hookworm(Ancylostoma duodenale), Trichuris trichiura, Ascaris lumbricoides and cysts of Blastocystis hominis, Entamoeba coli, Iodamoeba butschlii were observed. In addition, larvae of Strongyloides stercoralis were also detected.

Conclusion: The present case emphasizes the need to rule out multiple etiologies in underlying conditions of intestinal immune imbalance disorders like coeliac disease and others. These group of patients harbour more pathogenic parasites and are more frequently colonized with harmless commensals compared to healthy individuals. The cause and effect relationship of coeliac disease and parasitism still remains to be explored. Our report, therefore, emphasizes the need to look into parasitic infestations more precisely in other gastrointestinal disorders like Inflammatory bowel disease(IBD), Crohn’s disease and Ulcerative colitis to circumvent delay in the diagnosis and for institution of appropriate treatment.
Performance evaluation of malaria microscopists working at malaria slides rechecking laboratories for external quality assessment in Ethiopia

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Background: Microscopic diagnosis of Giemsa stained thick and thin blood films by skilled microscopists has remained the standard laboratory method for the diagnosis of malaria. The performance of Malaria Microscopists in all health facilities have been raised concerns by many experts. Microscopists who are working at Malaria Rechecking Laboratories have to be competent to cross check blood film slides which are collected from testing sites.

Methods & Materials: A cross-sectional study was conducted to assess the performance of 107 Malaria Microscopists who are working at 23 Malaria Rechecking Laboratories in Ethiopia. A set of 12 blood film slides containing known Negative and positive (different species, stage, and parasite density) results were distributed to each Malaria microscopists. Data was collected and entered into Microsoft Excel sheets and exported to software SPSS version 20 for analysis. Chi-square (for categorical data), sensitivity, specificity, percent agreement, and kappa score were calculated to assess laboratory professionals’ performance in detecting and identification of Plasmodium species. Association was taken as significant at P < 0.05.

Results: A total of 107 study participants were involved in this study, the mean age of the participants was 30+/-5.04 years. Overall, the sensitivity of participants in detection and species identification of malaria parasites were 96.8% and 56.7%, respectively. The overall agreement on detection and identification of malaria species was 96.8% (Kappa = 0.9) and 64.77% (kappa = 0.33), respectively. About 34(31.8%) participants were used unrecommended quantification (+) system. The least malaria species which were identified correctly by the participants were P. malariae(2.8%) followed by and P.ovale(32.7%). Malaria microscopists working at sub regional laboratory had a better Quantification performance (P=0.003). Study participants who were participated on malaria microscopy and quality assurance training had a better performance on parasite quantification (P<0.001).

Conclusion: Agreement of the participants with expert microscopist in the identification of different malaria species and quantification were very low. Most participants did not identify P. malariae and P.ovale correctly. Therefore, policy backed regular competency assessment and training for malaria microscopists is essential and mandatory that can assure proper diagnosis and management of malaria in Ethiopia.
Assessing geohelminth parasites among geophagous school children, in Owerri Metropolis Area, Imo State, South-Eastern Nigeria

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Background: Geophagia or soil-eating is a potential risky behavior, among other health hazards, associated with acquisition of geohelminth parasites, or soil transmitted parasites (STPs), especially among school-aged children.

Methods & Materials: A parasitological survey of geophagous children in four nursery-primary schools, in Owerri municipal council area, was investigated. A total of 300 pupils (170 females and 130 males) of age 3-14 years from nursery 3 to primary 6, were selected randomly, from the different schools and examined for geophagic practices and parasitic infections, with a well-structured questionnaire for Socio-demographic data and standard parasitological technique for detecting STPs. Anthropometric data and Stool samples were collected from them, with the help of their school health workers / nurses / class teachers, using clean dry, specimen bottles, and data-recording devices. All the collected stool samples were correctly labeled, transported to the laboratory and analyzed using concentration formol-ether technique and wet-mount microscopic examination.

Results: Results shows that, out of the 300 pupils examined, 25 (8.3%) were geophagic and 24 (8.1%) infected with parasites. The soil-transmitted parasites (STPs) observed include, *Ascaris lumbricoides* (33.3%), *Trichuris trichiura* (25.0%), *Entamoeba histolytica* (20.8%), *Strongyloides stercoralis* (12.5%), and Hookworm (8.3%). *Ascaris lumbricoides* was the commonest and most (33.3%) prevalent of all the STPs, while Hookworm was the least (8.3%). The presence of these geohelminth parasites in the study are statistically significant (P<0.05), among the younger children, within 3-6 years old. This younger age-group was the most geophagic (8.9%) and most infected (66.7), and highlighting a strong relationship, between geophagy and geohelminth parasitism. Also geophagia and parasitic infection decreased with increasing age, and were more pronounced in males (70.8%) than in females (29.2%).

Conclusion: These findings demonstrate that, geophagia is an important risk factor for orally acquired parasitic infections in school children. Therefore education regarding risk of geophagia in younger children and mass-targeted Chemotherapy should be an integral component of any STPs control programs, in nursery-primary schools in the studied area.
Pediculosis among school children, in Owerri north local government area of Imo State, South Eastern Nigeria

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**Background:** Social cultural orientation, poverty, lack of knowledge and poor environmental hygiene has continued to encourage the persistence of ectoparasites and infestations, especially among the most vulnerable school-age children leading to poor academic performance.

**Methods & Materials:** The study therefore, investigated the prevalence of lice infestation (pediculosis) among primary school children in Owerri North Local Government Area of Imo state, South Eastern Nigeria, between the months of June-September, 2015. A total of 500 (200 boys and 300 girls), of primary (1-6) school children, aged between 3-13 years, from four different schools, designated (A, B, C, and D), was investigated for the presence of lice infestation. A Questionnaire was used to assess the childrens’ and teachers’ knowledge, about the lice (*Pediculus humanus capitis* (head louse), *Pediculus humanus corporis* (body louse), and *Pthirus pubis* (crab louse)).

**Results:** Results show, that out of the 500 pupils examined, 14 (2.8%) were infested with the head louse only. This value was statistically significant (*p*< 0.05), among those infected, with a higher prevalence for girls 13 (92.9%) than boys 1 (7.1%). 90% of the child care givers agreed that, sharing the same bed with others, accounted for the prevalence of the ectoparasite. 30% advocated hand picking of the lice, as a preventive/treatment measure. 100% of the school teachers had good knowledge of pediculosis and agreed that, the infestation is preventable and treatable. Low socio-economic status pre-disposed the children to lice infestation arising from sharing beds, clothing and combs as reported by the enlightened teachers. 90% of the teachers mentioned lack of concentration as the major effect of pediculosis among the school children.

**Conclusion:** Preventive measures, such as, health education, personal hygiene, regular washing of hairs and use of hair cream containing sulphur, are therefore advocated for efficient eradication of pediculosis among the school-aged children.
Changing profile of malaria: An observational study in a central Mumbai hospital, India

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**Background:** Malaria has been one of the leading causes of acute febrile illnesses in India. A definite change in the trend of malarial infections, their clinical features and outcomes has been noticed recently. The present study, in a Mumbai hospital, during 3 consecutive monsoons, was carried out to observe and compare the changing profile.

**Methods & Materials:** An observational study was conducted at a hospital in central metropolitan Mumbai, India, during June to October, 2013 to 2015. Febrile patients, admitted from the medical outdoor and emergency departments, were tested by peripheral smear examination/malaria specific antigen. Other investigations included, total and differential counts, liver function tests (transaminases and bilirubin) and renal function tests (creatinine and urea). Hemodynamic instability (hypotension), thrombocytopenia with manifest bleeding, affected renal function either singly or in combination were the differentiating criteria towards critical care. The clinical, laboratory features and outcomes were compared.

**Results:** During 2013, of 41 diagnosed Malaria cases, 39 were P. Vivax and 2 mixed Malaria. 2014 saw a total of 55 Malaria cases, - 23 being P. Falciparum and 16 P. Vivax and mixed Malaria each. 2015 saw a surge in malarial infections, with 117 diagnosed cases, 107 being P. Vivax and only 10 positive for P. Falciparum. No mixed malarial infections were encountered. On comparison of laboratory and clinical features, during 2013, 7 (17%) presented with hypotension, 14 (34%) had transaminitis while 7 (17%) required platelet transfusion. During 2014, 4 presented with hypotension, 2 each, with bleeding manifestations, jaundice, renal dysfunction and altered consciousness. There was a mortality of 3. Despite the surge in Malaria cases in 2015, with severe anaemia in 98 patients, leucopenia in 77, transaminitis in 44, and thrombocytopenia in 45, platelet transfusion was required by one and all subsequently recovered.

**Conclusion:** Within the three consecutive years (2013 to 2015), it was observed that, Dengue has overtaken Malaria numerically, as a major cause of monsoon related febrile illness. Within the malarial infections, P. Falciparum appears to be on the decline. P. Vivax, has shown variability in clinical severity. Environmental circumstances that may have contributed need to be looked into.
Kala Azar patients management in a renovated SK Hospital, Mymensingh - A real experience
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Background: Visceral leishmaniasis (kala Azar) is still one of the major rural public health problems in Bangladesh. A cross sectional study was carried out to observe the pattern of Kala-azar patients admitted in SK Hospital Kala- Azar research centre(SKKRC).

Methods & Materials: The suspected kala azar patients from 2012 to 2014, either new case, treatment failure or with complication referred from different hospital were subjected for evaluation.

Results: SKKRC managed 267 cases in 2012, 382 in 2013 and in the year of 2014 total 428 patients. All cases are confirmed by RK-39 or positive LD body on splenic puncture or PCR. We diagnosed 7 patients with Tuberculosis, 10 patients with Hepatitis and 2 patients with Malaria co-infection. Besides numbers of Kala Azar patients presented with other comorbidities. One boy 9 year old got treatment every year in last 5 years in different regimen still positive for LD body. One patient, 12 year hailing from one endemic area with history of getting inj SAG, Miltefosine and Amphotericin at different time. Ten patients developed severe hypersensitivity reaction during treatment with Ambisome. Most of the patients that presented with PKDL had previously been treated for VL with SSG or tab Meltifosine. Recently few patients presented with PKDL after receiving Ambisome for VL treatment. Twenty patients had history of both SSG and Miltefosine treatment for Kala-azar in different time period developed PKDL. Three patients had history of successfully treated PKDL with inj SAG total 120 doses with apparent cure by disappearance of lesion again developed PKDL. Two baby only 2-3 year old diagnosed kala Azar with positive history of mother. One Kala Azar diagnosed pregnant lady delivered a term baby, but unfortunately both were died next day: fetal part placenta was found positive for LD body by PCR. Five patients were found both splenomegaly and PKDL.

Conclusion: Currently, treatment recommendations are usually based on data from endemic regions. There is no clear cut determination of treatment end point. Each species has a different sensibility to the different anti-leishmanial drugs. Therefore, in depth evaluation is needed for succession of national elimination programme.
Sporotrichoid papulo-nodules with Retiform rash: Unusual presentation of Leishmaniasis

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Background: Leishmaniasis is caused by intracellular protozoal parasites belonging to the genus Leishmania. HIV infection is an important factor for atypical presentation and widespread progression of visceral leishmaniasis.

Methods & Materials: A 54-yr-old Nepali male diagnosed with HIV infection in 1994 on HAART from 2012 with baseline CD4 count 90, complained of multiple dome shaped painful lesion over both hands since 12 months. He has received multiple blood transfusion for pancytopenia in last 3 years without any improvement in blood count. On examination he was emaciated, had pallor with generalised lymphadenopathy. He had distended abdomen with massive hepatosplenomegaly. Cutaneous examination showed multiple sporotrichoid dome shaped firm tender papules and nodules over bilateral hands with isolated nodules on nose, bilateral elbows, buttocks, ankles along with net-like violaceous to erythematous coalescing papules over bilateral legs and trunk.

Results: Punch biopsies from a nodule on hand and violaceous papule over the leg showed multiple intracytoplasmatic amastigotes within histiocytes on H & E and Giemsa stain. Bone marrow aspirate showed intra and extra-cellular LD bodies on Giemsa staining. Diagnosis of Visceral Leishmaniasis with cutaneous dissemination in a HIV-AIDS patient was kept. IV Amphoterecin 1 mg/kg/day was administered for 30 days along with blood transfusion. 1 month later patient followed up with partial resolution of skin lesions which showed persistent parasites and CD4 count remain below 100/mm3. In spite of HAART and anti-leishmanial therapy, no significant increase in CD4+ T-cells was observed. Patient died later.

Conclusion: In the setting of HIV, visceral leishmaniasis represents an opportunistic infection. Cutaneous localization is rarely described in AIDS and usually represents the primary site of infection, with a low number of lesions; however, a diffuse skin localization secondary to visceral dissemination of the protozoa is exceedingly uncommon.
Utility of Polymerase chain reaction in diagnosis of Acanthamoeba and Microsporidial keratitis

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**Background:** Acanthamoeba and Microsporidia are two opportunistic parasitic organisms which are now increasingly recognized as significant emerging cause of microbial keratitis. Early and accurate diagnosis is the most vital step in managing these infections as prognosis is directly related to timely diagnosis. Lack of clinical suspicion, clinical resemblance of the early stages to herpetic keratitis, cumbersome and expensive isolation techniques are some of the factors which makes the diagnosis more challenging. Current methods of diagnosis these parasites depends mainly upon their morphologic demonstration in the clinical specimen by microscopy. There is scope for diagnostic methods which are rapid, have high precision, specificity and sensitivity. PCR is a rapid and sensitive method for diagnosis and species identification of Microsporidia and Acanthamoeba.

**Methods & Materials:**

**Objective:**
Evaluation of the diagnostic utility of PCR in comparison to the conventional test.

**Design:**
Descriptive study

**Participants:**
All patients with suspected microbial keratitis presenting between October 2012 to June 2014 at the Ophthalmology OPD, JIPMER hospital.

**Methods:**
A total of 50 consecutive non-duplicated cases of keratitis were included in the study period of two years. All the samples were subjected to the conventional test like microscopy using Gram stain and modified trichrome stain, and PCR for Acanthamoeba and Microsporidia.

**Results:**
Mean age group of the patients in this study was 48.3 years and majority of them were females (54%). The predominant symptom with which the patients presented in our study was pain (60%). Corneal trauma with vegetative matter was a major risk factor accounting for 20%. Out of the 50 samples, 30% were bacterial keratitis and 16% were fungal keratitis. One (2%) of the specimens was positive for Acanthamoeba and two (4%) were positive for microsporidia by PCR, while, none of the specimens was positive by microscopy for Acanthamoeba and Microsporidia on Modified trichrome stained smears.

**Conclusion:**
Hence, this establishes the fact that PCR is superior to microscopy as it is a sensitive cum rapid method for the diagnosis of keratitis due to Acanthamoeba and Microsporidia.
Molecular cloning and production of type III Hsp40 protein co-chaperone PfZRF1 of human malaria parasite Plasmodium falciparum

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**Background:** Despite remarkable progress in combating malaria, this deadly disease still accounts for more than a half million deaths annually. In the light of growing incidences of drug resistance, an understanding of parasite biology is necessary for the development of new antimalarials. During life cycle in two different hosts Plasmodium falciparum experiences frequent thermal variations and physiological stress. Heat shock proteins (Hsps) are key players for its survival and making them attractive drug targets. Hence, present research emphasizes on *in silico* analysis, cloning and production of a type III Hsp40 protein, PfZRF1.

**Methods & Materials:** Orthologs of PfZRF1 were assigned by BLASTp and literature search. Domain architecture was drawn by SMART and Pfam. ClustalW and T-coffee were employed for multiple sequence alignment analysis. Phylogenetic tree was generated by NJ method using Phylip-3.695 for evolutionary relationship analysis. PfZRF1 and its domain constructs were amplified and cloned in pETM11 vector between NcoI, XhoI and Kpn1 restriction sites. His-tagged PfZRF1 and other domain constructs were expressed in *E. coli* B834.

**Results:** PfZRF1 orthologs were identified in 31 eukaryotes however found to be absent in prokaryotes. PfZRF1 is composed of a Hsp70-binding DnaJ domain and two DNA-binding SANT domains. An ubiquitinated histone H2A binding UBD domain was also identified based on human ortholog HsZRF1. As compared to HsZRF1, DnaJ domain has a 50 aas long parasite-specific insertion in loop region between helix II and III near Hsp70-binding HPD motif. Additionally, ribosome associating RAC_head domain was found less conserved with many insertions in PfZRF1. Phylogenetic analysis depicted PfZRF1 to be closer to unicellular eukaryotes. Full ORF (2820 bp) and several constructs covering different domains were amplified and cloned in pETM11. Different constructs were expressed after inducing with 0.5 mM IPTG at 16°C in *E. coli* B834 cells for soluble expression.

**Conclusion:** This study presents preliminary characterization of a type III Hsp40 co-chaperone of the parasite. Further functional characterization of this putative multifunctional chaperone would undoubtedly provide an insight in parasite molecular biology for the development of novel antimalarials.
Molecular evidence of *Bothriocephalus acheilognathi* (Cestoda: Bothriocephalidea) from India

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**Background:** *Bothriocephalus acheilognathi*, a tapeworm that infects a variety of fishes worldwide. This worm is native to East Asia and spread throughout the world due to aquaculture trade of ornamental fishes. Although, this worm has not been parasitize to any mammals, but recently, a study from Saint Laurent du Maroni (French Guiana) describes the first report of the egg isolation of *B. acheilognathi* from human stool, a case of accidental infection. However, this worm is also described from Northern part of India but currently there are no reliable data is available based on molecular studies in India. This study aimed to assess the molecular phylogenetic analyses of *B. acheilognathi* based on sequences of 18S and 28S ribosomal DNA and its distribution in India.

**Methods & Materials:** Specimens of *Bothriocephalus* were collected from *Xiphophorus hellerii*, a native of North and Central America. For morphology, worms were washed in saline and were fixed in 70% ethanol for further processing. For molecular study, a small fragment from the strobila was cut and stored in 95% ethanol until DNA extraction. Genomic DNA was extracted and ribosomal 18S and 28S were amplified and sequenced. The data were then analyzed using the Maximum Likelihood (ML) and Bayesian Inference (BI) algorithms.

**Results:** Data obtained from 28S and 18S gene shows close relationships with all the sequences of *B. acheilognathi* reported from other isolates of the same species available on the database. Although in both gene sequences, 28S shows more conserved in isolates of *B. acheilognathi*. In comparing to 28S, 18S gene shows deep phylogenetic relationships in *B. acheilognathi* sequences. In two different phylogenetic methods used for analyses of 28S gene, all the *B. acheilognathi* isolates were divided into three clades with the Indian isolate showed a close relationship with an isolate from South Korea along with other isolates of the same species from different geographical regions.

**Conclusion:** This study describes the molecular identification of *B. acheilognathi* from India. This study also highlights that low specificity of this cestode for a host can affect the native fish resources of India and can be a problem for adversely affecting a number of wild fish species.

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**Background:** Acute diarrhea remains a public health problem with a major cause of morbidity and mortality in children. The low middle countries continue to be the most affected areas in worldwide. The determination of the cause is the first step in right treatment management. Parasitic infections are common in children and are related to gastrointestinal disorders. Altogether, it has been established National Surveillance in Acute Diarrhea in children in Mozambique with the aim to determine the pathogens prevalent in this health condition. In this data we will only present information related to the parasitic infections.

**Methods & Materials:** From 2013 to 2015, a hospital-based surveillance was conducted in 5 hospitals of Mozambique. The surveillance is being conducted in children younger than 14-years, with acute diarrhea defined as three or more stools per day of decreased form from the normal, lasting for less than 14 days. Stool samples were examined for the presence of parasites using formol-ether concentration method and the Modified Ziehl-Neelsen staining technique. Laboratory results from children with information of age (in months) and gender were selected for this analysis. Statistics analysis were made using STATA version 12.1 package in 95% of confidence interval.

**Results:** A total of 597 children with acute diarrhea provided stool sample. Overall 64% (n=383) had completed information regarding age and gender. The median age of the children was 11 months (IQR: 8 to 16 months). Intestinal parasites species were prevalent, with *Ascaris lumbricoides* 18% (22), *Trichuris trichiura* 16% (20), *Entamoeba histolytica/ dispar* 12% (15), *Endolimax nana* 11% (13), *Entamoeba coli* 6% (8), *Giardia intestinalis* 2% (2) and hookworm 1% (1). *Cryptosporidium* sp. was the only opportunistic parasite detected in 34% (42) children. Infection with *Cryptosporidium* sp. was significantly associated with the presence of *E. histolytica/ dispar* ($\rho = 0.04$).

**Conclusion:** Soil-transmitted helminthes, *A. lumbricoides* and *T. trichiura*, are an important public health issue related to acute diarrhea in children. Detection of *Cryptosporidium* sp. was high and with the high rate of HIV infection in Mozambique, it can be consider important pathogen to take in account in diagnosis routine.
Trypanosoma cruzi infection in the heart of Colombian wild bats
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Background: Trypanosoma cruzi is a parasite protozoa that infects mammalians and in the human cause Chagas’ disease, which represent a major health problem in Colombia where an estimated of 436,000 individuals are infected, with 11% of the population at risk for contracting the disease. Moreover, the potential epidemiological significance of bats as possible reservoir hosts for T. cruzi, has been previously remarked. Different neotropical bats species have been reported to be susceptible to T. cruzi infection. They participate in important ecological processes and because of its ability to fly can spread infectious diseases from the natural environment to the homes of people. In Colombia, few studies on bats in endemic areas for Chagas’ disease have been performed. Thus, we evaluated the presence of T. cruzi in heart tissue taken from bats Cordoba department (northern Colombia), considered an endemic area for this infection.

Methods & Materials: 30 hearts of bats were collected in four rural localities from Cordoba department. The DNA was purified using a commercial high-purity PCR template preparation kit (Roche, Mannheim, Germany). The integrity of the purified DNA was analyzed through PCR amplification of the bat cyt b gene. PCR tests based on the TcH2AF-R and S35-S36 primers which amplify a fragment of SIRE element and a conserved region of minicircles from T. cruzi respectively, were evaluated for the detection of parasite in bats’ heart tissue

Results: A total of 11 samples (36.6%) of three localities were positives for both PCR. Three species were positive for the presence of T. cruzi: Carolia perspicillata and Dermanura phaeotis (frugivorous) and Molossus molossus (insectivore).

Conclusion: This is the first report of T. cruzi in the heart of naturally infected bats in Colombia. These findings imply that there is an active transmission of parasite among bats populations from Cordoba. Therefore, it is important to continue assessing how bats natural infection can be acquire and spread the parasite, since these species are highly distributed in the region and human intervention in their natural ecosystems is contributing the migration to urban areas, which increase parasite circulation in the disease domestic transmission cycle
A novel spiroindoline kills human malaria parasites via modulation of Na ion influx mediated autophagy and apoptosis

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**Background:** Malaria continues to be a global health burden, causing millions of death every year. Resistance to current antimalarial chemotherapy needs attention and demands for active drug candidates that can combat developing resistance mechanisms of Plasmodium. We synthesized natural product inspired scaffolds based on indoles from chiral bicyclic lactams as potential antimalarial compounds. The strategy involved site specific diversification of natural product scaffolds obtained from chiral bicyclic lactams, with discrete architecture and disparate stereochemistry with an attractive steps/scaffold ratio of 1.7:1. Upon screening of these scaffolds against *Plasmodium falciparum* 3D7 strain, two scaffolds with spiroindolone architecture showed low micro molar anti malarial activity. Furthermore, the most potent scaffold was investigated for its antimalaria activity at different concentrations *in vitro* using blood stage of *P. falciparum*. The concentrations of spiroindolones scaffold to reduce the growth rate by 50% and to kill the parasites were 19.62 µM and 17.93 µM, respectively. We observed the treatment of parasites with the lead scaffold induced Na influx along with an increase in intracellular calcium. Treated parasites showed PfATG8 - PfRAB7 co localization, an event that marks onset of autophagy in Plasmodium, mitochondrial membrane potential loss and DNA degradation a classical features of apoptosis. Parasites maintain stringent control over their ion concentration by expressing various channels and pumps to survive inside the host thus imbalance can be detrimental for the parasite growth. The observed cell death pattern in treated parasites might be outcome of the rise in intracellular Na+/Ca++ level caused by potent scaffold. Overall this study highlights the potential of spiroindolones scaffold for development of anti-parasitic compounds and its mechanism of parasite killing in eliciting a decent antimalarial response.

**Methods & Materials:** Parasite and mammalian culture Immunofluorescence assay TUNEL assay JC-1 staining Flow cytometry

**Results:** We observed changes in levels of sodium and calcium after treatment with our potent spiro scaffolds. Treated parasites showed typical features of autophagy and apoptotic death.

**Conclusion:** We report here synthesis of seven structurally and stereo chemically distinct scaffolds which were tested for their efficacy against the blood stage malaria parasite, *Plasmodium falciparum*. 
Subtype distribution of Blastocystis sp. isolated from children in Eskisehir, Turkey

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Background: To date, only a limited number of studies have investigated the pathogenicity of Blastocystis and its association with the subtypes in children. The aim of the current study was to investigate the prevalence of Blastocystis in children aged between 3 and 13 years with or without gastrointestinal complaints and to determine the distribution of the subtypes of Blastocystis.

Methods & Materials: A total of 303 stool samples were obtained from 84 children with diarrhea, who had been referred from two different pediatric emergency services, and randomly chosen 219 school children of 6 and 13 years of age from different socioeconomic environments and who did not have diarrhea. Stool samples were prepared with native-lugol and examined and then stained with trichrome and further examined under a light microscope.

Genomic DNA was extracted from the stool samples using QIAamp® DNA Stool Mini Kit. The extracted DNA samples were examined in terms of the presence of Blastocystis sp. using the real-time PCR method with the genesig® Standard Kit (Primer Design., UK) designed for the quantification of the Blastocystis G elongation factor-1 alpha gene. The PCR was performed using seven subtype-specific sequenced tagged site (STS) primers (SB83, SB155, SB227, SB332, SB340, SB336 and SB337) for the genotyping of Blastocystis sp. The collected data was analyzed using the SPSS (Version 17, Chicago IL, USA).

Results: 115 samples were found positive for Blastocystis sp.. Subtyping was successfully performed on 46 samples using sequenced-tagged site (STS) primers and the PCR method. The most frequently detected subtype was ST3 (43.4%) followed by ST1 (26.1%), ST4 (10.9%) and ST2 (8.7%). The mixed subtypes were identified in five samples (10.9%) as; ST1+ST3 (n=3), ST1+ST2 (n=1) and ST2+ST3 (n=1). None of the samples had ST5, ST6 or ST7. No statistically significant difference was found between the symptomatic and asymptomatic groups in terms of the Blastocystis sp. positivity and the distribution of subtypes (p> 0.05).

Conclusion: This is the first study conducted to investigate the subtype distribution of Blastocystis sp. in children in Turkey and the findings obtained from this study are in agreement with the related data available in Turkey.
Detection of Blastocystis sp. Infection using different investigation techniques in children with or without acute diarrhea

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Background: Blastocystis is a common human intestinal parasite with high prevalence in developing countries and one of the causative agent for acute infectious diarrhea. Microscopic methods such as native-lugol and trichrome staining are most frequently used in routine diagnosis. Our study to evaluate the prevalence of Blastocystis in children in Eskisehir, Turkey with or without acute diarrhea, using direct microscopy, trichrome stain and real-time polymerase chain reaction (PCR) technique.

Methods & Materials: Study was carried out between January 2011 and March 2013 in Eskisehir city center in children. They were admitted to the emergency unit with acute diarrhea and school children without diarrhea from seven different socioeconomic backgrounds have been enrolled. Stool specimens were investigated by routine fecal examinations in the Parasitology laboratory of Eskisehir Osmangazi University Education and Research Hospital. We investigated 961 stool samples taken from children. We have a limited number of molecular tests therefore 303 samples have been evaluated with these three methods. All of the symptomatic childrens stool samples (n=84) were chosen for molecular test and in asymptomatic children's stool samples (n=219) we were chosen by randomly selection.

Results: Blastocystis were seen in 38.6% of samples with direct microscopic examination, 35.6% of samples by the trichrome stain, 38.2% by PCR method. In symptomatic group respectively; 14.2%, 19.0% and 19.0% also asymptomatic group ratio respectively; 47.9%, 42.0% and 45.6%. In the symptomatic group, compared to PCR-based evaluation of Blastocystis infection, for direct microscopic evaluation the sensitivity was 12.5% and the specificity was 85.2%, while for trichrome staining 31.2% and 83.8% respectively while the negative predictive value was 80.5% and 83.8%, respectively. In the asymptomatic group, compared to PCR-based evaluation of Blastocystis infection, for direct microscopic evaluation the sensitivity was 74.0% and the specificity was 73.9%, while for trichrome staining 59.0% and 72.2%.

Conclusion: PCR is a useful technique for the evaluation of fecal samples in acute diarrhea for Blastocystis sp. Direct microscopic evaluation can be used to rule out Blastocytis infection in children with acute infectious diarrhea but there are needed multicenter and large-scaled molecular and clinical studies.
In vitro activity of different 5-nitroimidazole derivatives and essential oils against Trichomonas vaginalis

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**Background**: Trichomoniasis is a common sexually transmitted disease (STD) caused by *Trichomonas vaginalis*. Treatment of trichomoniais is usually achieved by 5-nitroimidazole derivatives. But some resistant strains to treatment failures of metronidazole have been reported and also numerous side effects, so it is continuing the search for alternative treatments. We evaluated in vitro effective concentrations of different 5-nitroimidazole derivatives and essential oils against *T. vaginalis*

**Methods & Materials**: *T. vaginalis* was grown in TYM medium which was supplemented bovine serum and Vitamin B12. The in vitro minimum lethal concentrations (MLC) and the time for drug efficacy were determined 48 hour cultured. The number of trophozoites were adjusted to 10^5 parasite/ml using hematocytometer. Metronidazoleand and two different ornidazole were prepared at concentrations 450 mg/ml. also standart carvacrol, *Origanum vulgare subsp.hirtum* oil and tea tree oil concentrations were prepared at concentrations 0.1ml/10 ml in steril saline solution. The activity of trophozoites was evaluated at 0-2-24-48 hours using trypan blue and compared to growth and effective concentrations((EC_{50}, EC_{90} and EC_{100}) were calculated

**Results**: All of the tree different 5 Nitroimidazole derivates commercial drugs were able to reduce %50 of the viable trophozoites after 24 hours with metronidazole at 0.78-1.56 µg/ml; while two different ornidazole at 6.25 µg/ml concentrations. In the essential oils, only *Origanum vulgare subsp.hirtum* was able to reduce 50% viable trophozoites in first 24 hours. There was over all little difference in the reduction of %90 of the viable trophozoites between ornidazoles which is 12.5µg/ml and 12.5-25µg/ml respectively though the %90 reduction of trophozoite viability was at 25µg/ml for metronidazole. Essential oils reduced %90 of the viable cells at concentrations between 50-100µg/ml. After 24 hours complete inhibition of viability (EC100) was at 25µg/ml for ornidazole while it was higher for metronidazole and ornidazole. *Origanum vulgare subsp.hirtum* essential oil inhibited the trophozoites totally at 50µg/ml; while the total inhibition of growth by tea oil was given after 48 hours at 100µg/ml.Carvacrol%100 inhibitory concentration was >100µg/ml.

**Conclusion**: These data suggest that *Origanum vulgare subsp.hirtum* oil may be a good candidate for treating trichomoniasis and that further investigation of this drug is warranted.
Increased transcriptional level of the H2-T23 (Qa1) and H2-Q7/Q9 (Qa2) genes during acute infection induced by two strains of Trypanosoma cruzi
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Background: Qa1 and Qa2 are non-classical MHC class Ib immunomodulatory murine molecules that exhibit high structural homology to their human functional homologs HLA-E and HLA-G, respectively. These molecules present restricted constitutive tissue expression, and little attention has been devoted to their role on infections. Methods & Materials: In this study, we analyzed by qRT-PCR the transcription profiles of genes encoding Qa1 (H2-T23) and Qa2 (H2-Q7/Q9) molecules in heart from BALB/c and C57BL/6 mice during Trypanosoma cruzi experimental acute infections induced by Y or CL strains. Results: Compared to non-infected mice, the heart expression of Qa1 and Qa-2 in BALB/c mice was 17-fold and 21-fold increased, respectively, while in C57BL/6 mice the transcription levels of Qa1 and Qa2 were 16- and 15-fold higher, respectively, during Y strain infection. For CL strain infection, the heart expression of Qa1 and Qa2 in BALB/c was 30- and 28-fold higher than the control group, respectively. In addition, the transcription levels of Qa1 and Qa2 was 24- and 25-fold increased in infected C57BL/6 mice compared to non-infected group. Conclusion: Taken together, both infected BALB/c and C57BL/6 mouse strains exhibited increased Qa1 and Qa2 heart expression, independently of the T. cruzi strain, and it was not related to resistance (C57BL/6) or susceptibility (BALB/c) to the acute T. cruzi infection. Considering that Qa1 and Qa2 are immunoregulatory molecules that inhibit NK and T CD8⁺ cells, the increased expression of these molecules may be interpreted as an attempt of the host to protect cardiac fiber destruction, controlling the inflammatory process, cytolysis and fibrosis.
Liver parasites of cattle slaughtered in Onitsha urban and environ, Southeast Nigeria

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Background: The rate of infection and the extent of damage of liver by parasites in cattle slaughtered in Onitsha and environ south east Nigeria were investigated from October to December, 2014. The question then was which parasites were involved and was there any economic loss as a result of the parasitic infections.

Methods & Materials: The study involved postmortem inspection on the slaughtered cattle. The livers were examined by making length wise incision on the ventral side, in such a way as to open up the gall bladder and the bile duct. Macroscopic changes in the liver were observed and their economic importance noted.

Results: Out of a total of 2010 cattle examined 273 (13.6%) were infected. Infection rates were 14.2, 13.6, and 13.2%, for the months of October, November and December respectively. Two types of flukes, Fasciola gigantica (12.0%) and Dicrocoelium hospes (1.1%) were identified along with hydatid cysts (0.5%). There were mixed infections of F. gigantica and D. hospes and also of F. gigantica and hydatid cysts. Infected liver showed thickening of the bile ducts and cirrhosis. In very heavy infections, the bile turned dark-green and more viscous than normal light green colour. The total weight of livers condemned by parasitic infection during the period was 675.7kg. Condemned liver due to F. gigantica was 524.5kg and that due to D. hospes was 133.7kg. A kilogram of liver was sold at $8, thus the total amount lost due to liver condemnation was $5,405.6.

Conclusion: The parasites found contributed to a remarkable economic loss due to liver condemnation. The nomadic management practiced by cattle rearers in Nigeria could aid infection. Very poor meat inspection facilities and uncooperative attitude of butchers were observed. Prompt chemotherapy of live animals is necessary. Restricting and feeding of the treated animals with hays before they are slaughtered is recommended.
Malaria and soil transmitted helminthes co-infection among Abia State polytechnic students, Aba, Southeastern Nigeria

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Background: Soil transmitted helminthes and malaria infections are among the endemic parasitic diseases that have caused over half a million deaths in most tropical parts of the world, where both have similar geographical distribution and co-infections are common. In Nigeria and other tropical countries, malaria and helminthes infections are reportedly endemic and pose significant health problems. It becomes very necessary therefore to determine the impact of malaria and helminthes co-infection.

Methods & Materials: Fresh stool and blood samples were collected from 400 students of Abia State Polytechnic, Aba, aged 18 years and above. The stool samples were analyzed using saline wet mount method. Blood was collected by finger prick to determine malaria parasitemia using thick film method. Univariate analysis and chi-square statistical tests were used to analyze the data.

Results: Out of 400 students sampled, consisting of 160(40.0%) males and 240(60.0%) females, 141(35.3%) and 72(18.0%) students were infected with malaria and intestinal helminthes respectively. The percentage co-infection was 53(13.3%). The females were more infected for both malaria (20.5%) and helminthes infection (10.8%) than males (14.8% for malaria and 7.3% for helminthes infection). However, the statistical analysis showed that co-infection of malaria and helminthes infections do not depend on sex. Species of soil transmitted helminthes isolated from the stool samples include *Ascaris lumbricoides* (4.5%), Hookworm (6.0%) and *Trichuris trichura* (4.8%).

Conclusion: Malaria and soil helminthes infection may co-exist without clinical symptoms, yet they pose serious health threats to the public. Interventions in the area of improved sanitation, drainage of stagnant water, health education and the need to sleep on insecticide- treated bed net are highly recommended.
Genome wide collation of zinc finger family in *P. falciparum*
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**Background:** Despite progress in recent years, malaria continues to be a major public health problem. *P. falciparum* - the most virulent species of human malaria requires specialized regulation of gene expression for survival in two different hosts. A subset of these regulators belongs to the zinc finger proteins (ZFPs) that are involved in transcription regulation, signal transduction, RNA binding and regulation of apoptosis. The present study identified the first genome-wide analysis of zinc finger family in *P. falciparum*.

**Methods & Materials:** All zinc finger genes were extracted from PlasmoDB v 9.0 by gene text search, BLASTp and HMM search. Domain architecture of extracted genes was drawn by SMART and Pfam. Orthologs in different organisms were assigned by BLASTp program. Evolutionary relationships were drawn using phylip and homology modelling was done by Phyre 2.

**Results:** In the present study, we identified 144 putative zinc finger genes in *P. falciparum*. It comprises almost 2.67% of all annotated genes present in plasmodium. Based on number and the order of the Cystine and Histidine residues that bind the zinc ion, ZFPs were classified into 16 different types. Nearly 70% (100/144) of these genes belong to four distinct classes namely, C2H2, C3H1, RING finger and PHD finger that play a critical role in transcription, RNA binding, ubiquitination and chromatin regulation respectively. Notably, the percentage of CCCH genes in apicomplexa (0.38% - 0.59%) was found to be much higher than that of chordates. Domain composition analysis revealed few domain compositions (RING+SPX) specific to the parasite as compared to the human host. Homology modeling revealed plasmodium specific insertions and residues as compared to its human homologue. Expression profiling of PIZFPs showed a mixture of linear and non-linear relationships between transcriptome and proteome and few stage specific proteins. Phylogenetic analysis of zinc finger showed good evolutionary relationships with high bootstrap values of the internal branches.

**Conclusion:** Presence of large number of ZFPs suggests an extensive mechanism of gene regulation in *P. falciparum*. The comprehensive genome wide analysis provides a solid platform for further investigations into the role of zinc finger proteins in development of parasite.
The level of Profilin and Interleukin-12 in obese patients infected by Toxoplasma gondii: A correlation study between Toxoplasma gondii infection and obesity

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Background: Background. Toxoplasma gondii is one of the microorganisms that cause chronic inflammation. It is also thought to be associated with obesity. Profilin T. gondii is an actin cytoskeleton protein with a molecular weight of 18 kD identified in the membrane of T. gondii. Interleukin-12 is a proinflammatory cytokine arising from the body's immune response to exposure to profilin. Profilin and IL-12 are thought to have a role in the pathomechanism of obesity.

Objective. The objective of the study was to determine the level of Profilin and IL-12 in obese individuals infected by chronic T. gondii and to examine a possible correlation between these levels.

Methods & Materials: Methods. We report an observational analytical study using a cross-sectional design. The subjects of the study were 57 obese individuals divided into a positive IgG T. gondii obese group and a negative T. gondii IgG obese group. Profilin, IL-12, and IgG levels were measured by ELISA. Differences between the two groups were examined using the independent samples t-test. Bivariate correlations between variables were examined using Pearson's test or the Mann-Whitney test. A value of p<0.05 was considered statistically significant.

Results: Results. The mean of profilin for the positive T. gondii IgG group was higher than that of the negative T. gondii IgG group (97.7±32.6 [SD] vs 64.4±25.1 µg/ml; p=0.002). The mean of IL-12 for the positive T. gondii IgG group was higher than that of the negative T. gondii IgG group (48.2 (range 22.14 to 117.21) vs 41.67 (22.70 to 51.14) µg/ml; p = 0.656).

There was no significant correlation between Profilin and IL-12 (r=0.056, p=0.677). However, there was a positive correlation between Profilin and IgG T. gondii (r=0.372, p=0.004).

Conclusion: Conclusion. The level of T. gondii Profilin and IL-12 are higher in the positive T. gondii IgG group than in the negative T. gondii IgG group, but there is no significant correlation between the level of Profilin and IL-12 in obese individuals. Further research is needed to know the pathomechanism of T. gondii profilin in causing obesity.
Comparative study between vivax and falciparum malaria in Eastern India: Breaking a myth
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Background: Plasmodium vivax has long been thought to cause benign infection which has been challenged recently. Various studies have pointed towards the rising severity of vivax infections. Till date no longitudinal study has compared vivax and falciparum malaria directly to show the final outcome. Our aim was to explore the manifestations of vivax and falciparum malaria infection and to follow them up and observe the final outcome over a period of 28 days.

Methods & Materials: In this hospital based longitudinal observational study, 89 patients attending a tertiary care hospital in Kolkata, who were positive for vivax or falciparum malaria (both complicated and uncomplicated)(confirmed by PCR) and who did not receive anti-malarial therapy from outside were consecutively recruited and their clinico-pathological, biochemical parameters and proteomics were analysed. They were followed up for a period of 28 days to observe their outcome after institution of appropriate guideline directed anti-malarial therapy.

Results: Of the 89 patients infected with either vivax or falciparum, 10 were complicated vivax and 12 were complicated falciparum. Jaundice and thrombocytopenia accounted for majority of complicated vivax whereas cerebral malaria, severe anaemia and thrombocytopenia accounted for majority of complicated falciparum infections. One of the vivax infection, complicated by jaundice and severe anaemia and two falciparum infections complicated by jaundice and cerebral malaria resulted in death. All the other patients showed improvement in their clinico-pathological and biochemical parameters over time. There was no statistically significant difference in the parameters over time when compared between complicated vivax and falciparum malaria. (p value>0.05). Of the multitude of protein changes in the serum of malaria patients, serum amyloid associated protein (SAA) and haptoglobin showed a characteristic pattern of change, with SAA being upregulated and Haptoglobin being downregulated; the alterations in these two protein levels being much higher in the complicated vivax and falciparum infection than uncomplicated infection.

Conclusion: Physicians should give equal importance to vivax as a cause of severe malaria as falciparum, with thrombocytopenia and jaundice being the most common complications. Policy makers should consider giving equal importance to severe vivax as severe falciparum and make amends in their action plan against malaria.
Assessment of malaria transmission intensity using anti-MSP1.19 (Plasmodium vivax) antibody as a serological marker in a previously malaria endemic district in Sri Lanka
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**Background:** Serological markers have been identified as good indicators of malaria transmission intensity, including in elimination settings. This study assessed the ability of using anti-malaria antibody MSP1.19 for vivax malaria to predict changes in transmission intensity in a previously malaria endemic district in Sri Lanka i.e. Kurunegala.

**Methods & Materials:** Serum was collected from 637 individuals (469 females: 150 males) and was subjected to standard ELISA to determine the sero-positivity for anti-malarial antibody MSP1.19 (P.v). Sero-conversion rate (λ) and sero-reversion rate (ρ) were estimated by fitting the optical density values obtained using ELISA to a simple reversible catalytic conversion model.

**Results:** Age ranged from 1 – 84 years (mean=43.31 yrs, median = 46 yrs). Study participants were grouped into 4 age groups i.e. 1-5 years, 6-10 years, 11-20 years and >20 years. Previous exposure to malaria was low (18.8%) and the number of individuals with past history was significantly high in 11-20 and >20 years categories when compared to the younger age groups. Over 60% of the population was sero-positive for MSP1.19 (P.v.) antibody. Sero-prevalence did not significantly differ between the District Secretariat divisions, nor between males and females. The number of sero-positive individuals below 10 years were significantly lower than the expected counts, while the number of sero-positive individuals were significantly higher than the expected counts in11-20 and >20 year age groups. The association between sero-positivity and malaria exposure was relatively poor and not significant. The age specific sero-prevalence was fitted to a simple reversible catalytic model using maximum likelihood method. The estimated annual sero-conversion for the particular area (λ² = 0.011/year) indicated that the transmission intensity is very low in the Kurunegala. This was significantly lower when compared to the sero-conversion rates 30 years ago where the sero-conversion rate (λ₁) =0.101 and sero-reversion rate (ρ) =0.030.

**Conclusion:** The maximum log likelihoods indicated that a reduction in P. vivax transmission intensity occurred approximately 30 years ago in Kurunegala district, Sri Lanka.
In-silico analysis of Chromatin Assembly Factor 1 (CAF-1) family and production of PF3D7_0110700 protein in human malaria parasite Plasmodium falciparum

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Background: Chromatin Assembly Factor 1 (CAF-1) is a histone chaperone that promotes DNA synthesis-coupled chromatin assembly during DNA replication and DNA repair. It is a highly conserved heterotrimeric protein complex basically required for normal S-phase progression and chromatin restoration during DNA repair. Here, we report a comprehensive In-silico analysis of PfCAF-1 family and cloning, production and purification of PfCAF-1 gene (PF3D7_0110700).

Methods & Materials: PfCAF-1 genes sequence was retrieved from PlasmoDB and domain architecture was drawn by SMART and Pfam. Orthologs in different organisms were identified by BLASTp program. Multiple sequence alignment of PfCAF-1 was done by ClustalW. PfCAF-1 (PF3D7_0110700) was cloned in two vectors (pTEM11 & pTEM30) and expressed in E. coli B834 cells. Recombinant protein was purified by Ni-NTA chromatography. Polyclonal antiserum was raised in New Zealand White rabbit.

Results: In the present study, five PfCAF-1 genes were identified. Out of five genes, four posses CAF1C_H4-bd and WD40 domain composition while one contains only WD40 repeats. Largest subunit of CAF-1 complex was found to be missing in P. falciparum. Analysis of protein expression profiles revealed three genes to be trophozoite specific at both mRNA and protein level, whereas, other two showed nonlinear relationship between transcriptome and proteome. The modelled 3-D structure of PF3D7_0110700 depicts the conserved H3-H4 binding pocket. However, multiple sequence alignment showed the variation in five residues that are important for histone binding as compared to its human homologue. PF3D7_0110700 was amplified, cloned and expressed in E. coli with His tag and GST tag. When induced with 0.25 mM IPTG at 16°C, we were able to get the protein in soluble form. The recombinant protein was purified by Ni-NTA chromatography. Raised polyclonal antibodies were able to detect native protein.

Conclusion: The present study provides a detailed description of CAF-I family in P. falciparum. The absence of the largest subunit suggests the presence of some different mechanism for chromatin assembly. The purified recombinant protein could be exploited for further functional characterization.
Effect of Valeriana officinalis hydroalcoholic extract on Giardia lamblia cysts

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Background: Giardia lamblia is an important and prevalent parasitic cause of diarrhea and gastroenteritis. Regarding the significance of giardiasis treatment particularly by medicinal plants and G. lamblia resistance to chemical drugs, this study was conducted to study in vitro effect of Valeriana officinalis hydroalcoholic extract on G. lamblia cysts.

Methods & Materials: In this experimental, laboratory study the hydroalcoholic extract of V. officinalis at concentrations of 12.5, 25, 50, 100, and 200 mg/mL was applied on G. lamblia cysts. The findings were compared with contr.

Results: Mean results of the effect of V. officinalis hydroalcoholic extract at different concentrations on G. lamblia cysts after 1, 6 and 24 hours demonstrated that the extract at all concentrations caused a notable decrease in alive cysts, with more intensive effect at 100 and 200 mg/mL concentrations and 100% fatality after 1 hour. As the extract concentration decreased, the speed of G. lamblia cysts inhibition declined.

Conclusion: V. officinalis hydroalcoholic extract might be recommended as an effective compound for removing G. lamblia protozoan cysts, although further studies are needed to show this effect on human.
Isospora belli associated recurrent diarrhea in a patient with AIDS
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Background: Recurrent diarrhea is a major complication of Acquired Immunodeficiency Syndrome (AIDS) or Human Immunodeficiency Virus (HIV) infected cases. Isospora belli causes diarrhoea in patients with AIDS. The prevalence of isosporiasis is probably underestimated in developing countries because routinely not all HIV-infected people are examined for the presence of this protozoan infection.

Methods & Materials: Here we report a case with AIDS related isosporiasis presenting with failure to thrive and recurrent diarrhea. A 27 year old HIV-infected male with a CD4 count of 118 cells/mm³, HIV viral load was 70729 copies/ml presented with 3 months of watery diarrhea. In the last two year period, Isospora belli oocytes were found in multiple stool samples examination with modified acid-fast staining for 4 times intermittently (Figure 1).

Figure 1

Results: At last time, his CD4 count had risen to 163 cells/mm³ and HIV viral load was <50 copies/ml from 12 months of antiretroviral therapy (ART). Three years after his first presentation, he is still suffer from repeat episodes of mild to moderate diarrhea.

Conclusion: Careful screening of HIV infected individuals is essential for early treatment of Isospora belli infection. Furthermore, raising immune status of HIV infected individuals with ART may help to decrease acquisition or proliferation of HIV related parasitic infections and the likelihood of experiencing diarrhea. Immunodeficiency increased the risk of having opportunistic parasites and diarrhea. Therefore; raising patient immune status and screening at least for this treatable parasite is important.
Association of interleukin-18 gene polymorphism with susceptibility to visceral leishmaniasis in endemic area of Bihar, an Indian population

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**Background:** Interleukin 18 (IL-18) is a cytokine that mediates Th1 response by inducing interferon-gamma (IFN-γ) production in T cells and natural killer cells. Genetic polymorphisms in the IL-18 gene have been found to be associated with its expression in cancer, tuberculosis, HBV infection and various other diseases. Lower plasma level of IL-18 in visceral leishmaniasis patients might be associated with polymorphisms in the coding gene.

**Methods & Materials:** Three single nucleotide polymorphisms, rs1946519 (-656 G/T) and rs187238 (-137 G/C) in the promoter region and codon 35/3 (+105 A/C) were genotyped in 204 parasitological confirmed visceral leishmaniasis (VL) patients and 267 controls with no past history of VL. For each locus, polymerase chain reaction followed by restriction digestion was performed. IL-18 expression in peripheral blood mononuclear cells collected from VL patients and controls was measured by quantitative real time RT-PCR.

**Results:** Distribution of G allele at position -656 ($P<0.0001$) and double haplotypes GGC/GGA ($P=0.05$) were found to be significantly associated with controls while genotypes TT ($P<0.0001$) and single haplotypes TGA ($P=0.0002$) were significantly associated with cases.

**Conclusion:** The inheritance of G allele at the position -656 might be considered as a genetic factor for resistance to VL.
Detection of entamoeba dispar and entamoeba moshkovskii DNA in liver abscess pus: Newer perspectives to be considered in diagnosis of amoebiasis

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Background: Amoebiasis, caused by *Entamoeba histolytica* is the third common parasitic cause of morbidity & mortality. Its prevalence in the global scale is 2-60% whereas in the Indian scenario is 3.6 – 47.4%. To further aggravate the clause, specificity of the available diagnostic assays is uncertain, thereby foiling the early diagnosis. Hence, it remains a serious public health problem even today. Molecular tools aid in resolving the issues related to the diagnosis, epidemiology and clinical co-relation of *Entamoeba* species without the need for culturing the parasites. Analysis of the small subunit (SSU) rRNA gene sequence data provides insights into the phylogenetic relationship and genetic diversity of the different *Entamoeba* species. This study aims at differential detection of the *Entamoeba* species from liver abscess pus samples.

Methods & Materials: A total of 115 Liver abscess pus samples were subjected to conventional microscopic examination and nested-multiplex PCR assay targeting the SSU rRNA gene for differential detection of *E. histolytica*, *E. dispar* and *E. moshkovskii*. The PCR products were analysed by agarose gel electrophoresis and further confirmed by sequencing.

Results: A total of 53 samples were found to be positive for *E. histolytica*. The presence of *E. dispar* with *E. histolytica* DNA was observed in 2 samples whereas, *E. moshkovskii* DNA along with *E. histolytica* was observed in 5 samples. The sequencing results were on par with that of PCR. At the outset, this finding leads us to think over different hypothetical scenarios to reconsider the pathogenicity of the said to be non-pathogenic species.

Conclusion: The ability of *E. dispar* to cause liver abscess pus in human is unclear; however, hepatic lesions in animals produced by experimental induction of *E. dispar* and presence of *E. dispar* DNA from liver abscess pus samples have been reported previously. But till date, presence of *E. moshkovskii* DNA in liver abscess pus samples has not been demonstrated. And hitherto, the pathogenicity of both *E. dispar* and *E. moshkovskii* are under question. This report on the detection of *E. moshkovskii* from liver abscess pus samples will open up newer perspectives to be considered in the diagnosis of amoebiasis.
Development of Glycine coated Magnetic Nanoparticles (GMNPs) advance drug delivery system against visceral leishmaniasis

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Background: The treatment options for visceral leishmaniasis (VL) caused by Leishmania donovani are limited and unsatisfactory at present. The drugs available are mostly parenteral and have serious side effects. Development of glycine coated magnetic nanoparticles as a based nanocarrier has been carried out which can enter into macrophages and deliver antileishmanial drug to target site of macrophage.

Methods & Materials: We have developed the MNPs drug target system against visceral leishmaniasis. The coating of Fe₃O₄ nanoparticle has been done with glycine was carried out in situ during co-precipitation of Fe²⁺ and Fe³⁺ ions in basic medium. The terminal amino acid on the shell of the magnetic nano carriers allows us to create functionalized exteriors with high densities of organic moieties (both amine and carboxyl) for encapsulation of drug molecules. Synthesis and size confirmation of GMNPs-AmpB has been carried out by different sophisticated instruments such as XRD, FTIR, TEM, DLS-Zeta, VSM, TGA and CHNS.

Results: Synthesis of Fe₃O₄ nanoparticle has been done with coating of glycine. Size of nanoparticle showed that particle size in 10-15 nm and closed to spherical in shaped. Furthermore 90% loading affinity of GMNPs for AmpB drug and their Higuchi drug release model confirming that the AMP release process is diffusion controlled, release rate of AmpB is higher at lower pH. During in vitro study, efficacy of GMNPs encapsulated AmpB formulation against conventional AmpB IC50 value are 4 ng/ml and 9 ng/ml was observed for nano-AmpB and AmpB respectively, significantly more than two fold efficacies of GMNP encapsulated AmpB has been increased. There was a highly significant reduction in the total parasite burden in spleen in the treated groups, 94.53% parasite inhibition has been seen with GMNPs encapsulate AmpB which is showing very high % as compare to AmpB with 75.73% parasite inhibition.

Conclusion: The studies, therefore, could provide another useful tool for successful development of GMNPs and an in vitro and vivo approach to designate nanocarriers system with distinctly improved bioavailability, high efficacy, and premature degradation of drug and to overcome side effect of antileishmanial drug.
ITS Typing, a potent genetic tool for discrimination of Trichomonas vaginalis isolates
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Background: Genetic variability, virulence factors and drug resistance mechanisms of Trichomonas vaginalis are poorly understood issues about the parasite. The aim of this study was to determine internal transcribed spacer (ITS) types of available T. vaginalis ITS sequences in Genbank and to compare with ITS types of T. vaginalis isolates from Aydin, Turkey.

Methods & Materials: T. vaginalis isolates in the present study were obtained from 20 patients with vaginitis at Adnan Menderes University, Research and Training Hospital Parasitology Department between 2010 and 2014. ITS regions were amplified and sequenced from a total of 20 T. vaginalis isolates.

Results: The sequences were compared and over 99% homology was observed. Of 20 isolates 5 were identical to the reference which was defined as ITST1. A total of 13 strains had A58 deletion (ITST10), and one strain had C203T mutation (ITST2), and one strain had both A58 deletion and C203T mutation (ITST11). ITS typing of 34 T. vaginalis ITS sequences on Genbank was also done and a total 11 ITS types including 2 from present study were defined. Our study showed that ITST10 was the most common ITS type in Aydin, Turkey. Based on sequences in Genbank the ITST1 was the most common type in the world (44.4%), and isolates were reported from different countries.

Conclusion: ITS typing is an important tool for molecular epidemiology analysis and for determination of dissemination of T. vaginalis clones.
Biological pollution of drinking water ponds (hafirs) with toxoplasma gondii, giardia and cryptosporidium spp in Eastern Sudan

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Background: Many classes of pathogens excreted in animals and human feces are responsible for waterborne disease. Protozoans are known to cause outbreaks disease worldwide and they are very robust in water environments and are strongly resistant to most disinfectants, including chemical procedures. Access to adequate supplies of good quality drinking water continues to be limited among many nomadic communities in Sudan where the major source of water, for both humans and livestock, is a rain-fed natural surface reservoir (hafir). The purpose of this work was to investigate the occurrence of oocysts and/or cysts, including those of Toxoplasma gondii, Cryptosporidium spp., and Giardia, which are the main parasites associated with waterborne diseases.

Methods & Materials: Water samples from 21 stagnant small ponds (hafir) at Eastern Sudan, were collected, from October to March 2014, in sterile containers. Conventional detection of Giardia and Cryptosporidium spp relies on filtration, cyst concentration, purification, and detection steps by Fluoresceinated monoclonal antibodies. After that the eluate was centrifuged, the supernatant discarded and the pellet suspended in a sucrose suspension (density 1.15). After another centrifugation, 2 ml of this supernatant was collected and mixed with 8 ml of de ionized water, then, a final centrifugation was performed in order to collect sediment for Toxoplasma oocyst detection. Toxoplasma oocyst was confirmed by mice bioassay after sporulation in 2.5% potassium dichromate in an aerated tube for 7 days. ELISA test was carried out on the surviving mice, an autopsy was carried out on mice and tissues were fixed in 10% formalin for histopathology.

Results: T. gondii oocysts were detected in 17 water samples, Cryptosporidium oocysts in 9 samples and Giardia cysts in 15 samples. Infection was documented in all surviving mice by positive serology (ELISA) results. Toxoplasma tachyzoites were observed in the mice specimens. There was no correlation between T. gondii presence in environmental water and the presence of Cryptosporidium or Giardia

Conclusion: The present study investigated fecal water pollution at the point of consumption among nomadic pastoralists communities and also showed that poor quality water continues to be a major risk factor for public health in Eastern Sudan communities.
Genotyping of acanthamoeba spp causing granulomatous amoebic encephalitis
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Background: Granulomatous amoebic encephalitis (GAE) is a serious human disease that predominantly occurs in immunocompromised individuals. It can be caused by two protozoan pathogens, Acanthamoeba spp and Balamuthia mandrillaris. Acanthamoeba GAE is a rare infection; it almost always proves fatal with the mortality rate of more than 90% and majority of GAE infections are identified at the post-mortem. Previous studies have reported association of some genotypes with increased virulence. The aim of our study was to genetically characterize the Acanthamoeba isolates causing GAE.

Methods & Materials: A total of 5 Acanthamoeba isolates obtained from cerebrospinal fluid of GAE patients which are being maintained in monoxenic culture on Non-Nutrient agar were included in the study. Genotype identification was carried out with a PCR based on sequence analysis of the 18S rRNA gene. The genus-specific primers JDP1 and JDP2 were used for the amplification of Diagnostic fragment3. Direct sequencing of PCR products were performed with the conserved primers 892C. Genotypes were identified by Blast search and phylogenetic analysis.

Results: Acanthamoeba strains recovered from the GAE patients were identified as T4 and T11 genotypes. DNA sequence analysis of the 18S rRNA gene shows 3 isolates belonged to T4 genotype and 2 isolates belonged to T11 genotype. To the best of our knowledge, there are no reports of Acanthamoeba genotyping involving GAE in India.

Conclusion: Acanthamoeba is ubiquitous in nature. GAE occurs as an opportunistic infection in immunocompromised hosts. T4 is most common genotype found in clinical and environmental isolates.
Zoonotic parasitic diseases at human-animal interface: a comprehensive study at a Zoological Garden in Punjab, India

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**Background:** Parasitic diseases of public health concern at the wildlife-human interface are of particular importance in zoological gardens, especially humans (veterinarian and zoo-keepers) coming in close contact with the captive wild animals. With the increased contact, the risk posed by multi-host parasites for humans and wildlife populations increases and the chances of ‘spill-over’ and ‘spill-back’ infections become of utmost concern.

**Methods & Materials:** A two year long comprehensive study was carried out to assess the parasitic infections of the wild animals, kept at MC Zoological Park, Chhatbir, Punjab, India, by employing classical, molecular and serological parasitological techniques. The whole study involved the screening of 909 scat samples of animals for the assessment of gastrointestinal parasitism by using concentration and sedimentation techniques. Molecular and serological techniques were employed for the confirmation of zoonotic parasites involving: *Toxoascaris leonina* (in Asiatic lions), *Baylisascaris transfuga* (in Sloth bear), *Trichuris* species (in non-human primates) and *Toxoplasma gondii* (in captive wild felines), respectively.

**Results:** The scat samples screening revealed the gastrointestinal parasitic infection of 25.52% (95% CI= 23.08-27.97%) in captive wild animals. But, the most common zoonotic parasites encountered by classical parasitological techniques involved: *Toxocara canis* in hyena, *Baylisascaris transfuga* in bears, *Toxascaris leonina* in Asiatic lions, *Strongyloides fuelleborni* and *Trichuris* species infections in non-human primates, *Spirometra* species in captive wild felids including jungle cat, leopard cat and leopards. The molecular assessment confirmed the presence of *Toxascaris leonina*, *Baylisascaris transfuga* and *Trichuris* species in non-human primates. Serological studies revealed presence of toxoplasmosis in Asiatic lions (2) and tigers (3).

**Conclusion:** The present study highlights the presence of zoonotic parasites in the zoological garden, which establishes the vulnerability of contracting the infection by humans at contact. Further, studies are warranted on human population (zoo-keepers and veterinarians) of the zoological garden for the assessment of parasitic invasion representing the ‘spill-over’ infection and possibility of the captive wild animals of contracting the infection from them (spill-back infections). Further study will accomplish the necessity to generate a clear picture of transmission of parasitism at human-animal interface in the zoological garden.
An oral formulation of Amphotericin B for the treatment of visceral Leishmaniasis: f-Gr-AmB

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Background: The oral administration of Amphotericin B (AmB) has a major drawback of poor gastrointestinal solubility and permeability. The aim of this study was to investigate the potential of functionalized graphene as a nanocarrier to improve the oral efficacy of AmB. Antileishmanial activity was determined in vivo in hamsters to investigate its therapeutic use.

Methods & Materials: Animals and Parasites
Male Syrian golden hamsters, *Mesocricetus auratus*, (45–50 g) were procured from the animal house facility of the Central Drug Research Institute (CDRI), Lucknow were used as an experimental model for raising leishmania infection to assess the *in vivo* antileishmanial activity. *L. donovani*, LEM138 (MHOM/IN/00/DEVI) stationary stage promastigotes were used for *in vivo* work.

Results: The experimental observation from the preliminary data of *in vivo* experiments evoked a linear relationship in reduction of parasite burden to dosage of the novel formulation. The f-Gr-AmB administered orally at a dose 5 mg/kg and 10 mg/kg body weight for five days of treatment resulted in 72% and 89% respectively compared with control group that received PBS. Intra peritoneal administration of f-Gr-AmB at 5 mg/kg resulted in 91% parasite inhibition. Further, at the oral dose of 10 mg/kg, f-Gr-AmB has a significantly greater antileishmanial activity than 5mg/kg Miltefosine, the only oral drug available for VL. Antileishmanial activity of single dose intraperitoneal treatment of Ambisome resulted in the 98 % inhibition of spleen amastigote parasites, when it was administered at a dose of 5 mg/kg body weight. The antileishmanial activity of intraperitoneal f-Gr-AmB was superior to Miltefosine which has shown 78 % inhibition in the splenic parasite burden, when it was given orally to hamster at a single dose of 5 mg/kg body weight.

Conclusion: These results suggest that amine modified graphene could facilitate the oral delivery of AmB.
Study of profile of Plasmodium vivax malaria in a medical college hospital
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Background: Malaria is a devastating parasitic disease transmitted through the bite of infected female anopheles mosquito. Complicated life threatening malaria is usually caused by plasmodium falciparum, but the incidence of complicated malaria caused by plasmodium vivax is on the rise. A general shift in the clinical profile of plasmodium vivax with multiorgan dysfunction is becoming common. The anti malarial drug policy suggests that all plasmodium vivax cases should be treated with chloroquine in full therapeutic doses. ACT (Artesunate+Sulphadoxine/Pyrimethamine) is advised for chloroquine resistant and complicated cases.

Objective: To study incidence of vivax malaria, clinical profile and course of the disease.

Methods & Materials: All cases of confirmed malaria, clinically diagnosed as malaria and treated with antimalarials were analysed. This study was done at PESIMSR, Kuppam.

Results: A total of 170 cases of malaria were analysed of which 35 were complicated malaria. Among them 10 were vivax positive while 10 were clinically started on antimalarials, while remaining 15 were falciparum positive. Of the remaining 135 cases, 95 were clinical malaria diagnosed on the basis of presentation of fever with chills and splenomegaly, and other causes of fever were ruled out by relevant investigations. These patients were started on chloroquine or/and ACT depending on the severity. Of the remaining 40 cases, 16 were positive for vivax, 8 for both vivax and falciparum. Thus 46 were positive for vivax (20%) compared to falciparum (22.94%) among the confirmed cases, thus confirming an increasing incidence of vivax malaria in recent times. Mortality was 2 (1.17%) of the total vivax cases.

Conclusion: Malaria was positive in 38.23% of the total cases treated for malaria and the remaining were clinically/therapeutically proven malaria cases. This shows that vivax malaria is increasing in areas of endemicity and same importance has to be given in managing the cases similar to falciparum. Clinically proven malaria patients had palpable spleen as a clinical sign in suspected cases of malaria along with fever with chills. High index of suspicion and early initiation of treatment helps in good recovery of patients.
Prevalence and drivers of human scabies in Cameroonian prisons

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Background: There is dire lack of data regarding the epidemiology of human scabies (HS) in sub-Saharan African prisons, especially in Cameroon, a Central African country. The purpose of this study was to assess the prevalence and drivers of HS in prisons of the West Region of Cameroon.

Methods & Materials: From June to August 2014, 755 volunteer inmates were consecutively recruited in three randomly selected prisons of the region. Each participant underwent a clinical assessment independently conducted by two experienced and well-trained dermatologists. The diagnosis was ascertained if the patient complained of pruritus, especially prevailing at night, with pruritus in the entourage and/or contamination, associated with the presence of specific or non-specific lesions of HS, peculiarly found at meaningful body sites. Logistic regression analyses served to identify the drivers of HS.

Results: Ages of participants ranged from 14 to 82 years with a mean of 32 ± 12 years. There were 17 (2.3%) women. Overall, 242 cases of HS (32.1%; 95% confidence interval (CI): 28.8-35.4%) were recorded. Duration of incarceration was significantly lower among prisoners with scabies than in those without (p = 0.006). The number of detainees sharing their clothes/bedding (197/242 vs 278/513) and the number of detainees per cell (median 25 vs 17) were significantly higher in infected prisoners when compared to those non-infected p<0.0001). In multivariable logistic regression analysis, low educational level (adjusted odds ratio (aOR) 1.9, 95% CI: 1.4 – 2.7; p<0.0001), sharing of clothes/bedding (aOR 2.7, 95% CI: 1.8 – 4.1; p<0.0001), and number of detainees per cell >10 (aOR 1.9, 95% CI: 1.3 – 2.8; p = 0.002) were the three independent factors significantly impacting the occurrence of scabies. Age, sex, duration of incarceration, number of baths per week and number of washing per week were not associated with HS.

Conclusion: Human scabies is very common in prisons of the West Region of Cameroon. Low educational level, sharing of clothes or bedding and number of detainees/cell > 10 are independent determinants increasing the likelihood to develop scabies in these milieus.
Trichomonas vaginalis infection and reproductive complications in women from central Iran

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Background: Trichomoniasis infection is the most common non-viral sexually transmitted disease worldwide. In this study, the frequency of Trichomonas vaginalis infection and the possible risk factors were examined in women attending gynecological clinics in Qom, central Iran.

Methods & Materials: A total of 300 women aged 18-50 years were enrolled. Three swab specimens were collected from vaginal discharge of each woman. One swab was used for wet mount preparation and examined for flagellated organism with motility by microscopy; the second swab was used for smear preparation and stained with Giemsa and Gram and examined under the light microscope for cellular/bacterial morphology. The last swab was inoculated into PBS, DNA was extracted by phenol-chloroform method and PCR was set for ITS DNA region. The result of ITS-PCR was confirmed by sequencing of the PCR product.

Results: Of total 300 vaginal specimens, 34 samples (11.3%) in wet mount and 96 samples (32%) in ITS-PCR were positive for T. vaginalis (T.V.) infection. The result of sequencing showed the target gene was amplified. Chi-Square and Fisher-Exact tests showed statistically significant difference between T.V. positive and T.V. negative subjects in regard of dysuria (P=0.001), itching (P=0.05), history of abortion (P=0.001), low-birth weight (P=0.05) and ectopic pregnancy (P=0.002). There was also a significant increase in epithelial cells and white blood cells (P=0.000) and Gram+ bacteria (P=0.001) shedding in vaginal discharge of T.V. positive group. Binary logistic regression analysis showed that the risk of T.V. infection was significantly increased in women with history of abortion (OR=91.84; 95%CI=15.50-544.22; P=0.000), premature birth (OR=43.29; 95%CI=2.78-671.98; P=0.007), and PROM (OR=21.75; 95%CI=2.12-222.95; P=0.009).

Conclusion: Awareness should be raised in women with T. vaginalis infection regarding the high risk of reproductive complications such as abortion. Early diagnosis by PCR and accurate treatment of infected people could prevent dissemination of the infection.
Molecular approach to detect Albendazole resistance in Trichuris trichiura among Orang Asli in Malaysia

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**Background:** Albendazole (ABZ), a benzimidazole (BZ) drug is commonly used to treat gastrointestinal parasites (GIP), mainly soil transmitted helminths (STHs) like *Trichuris trichiura* and *Ascaris lumbricoides*. Mass drug administration (MDA) has been used for the control of these infections but parasite anthelmintic resistance has been reported in human. Single nucleotide polymorphism (SNPs) in the β-tubulin gene at codon positions 167, 198 and 200 is reported to cause resistance in parasitic nematodes. Benzimidazole resistant study by screening β-tubulin gene has not been investigated in Malaysia. Thus, in this study, we investigated the presence of resistance markers at codon 198 and 200 of β-tubulin gene in *Trichuris trichiura* before and after 1 month of albendazole administration.

**Methods & Materials:** This study was conducted at Kg. Serendah, Malaysia. Genomic DNA was extracted from individual eggs in fecal samples pre and post albendazole treatment. A simple conventional PCR using high fidelity enzyme was used to amplify β-tubulin gene from *Trichuris trichiura*. The PCR products were sequenced using Sanger sequencing method. The sequences obtained were aligned using Vector NTI 9.0™.

**Results:** Post 1 month treatment, the cure rate for *Trichuris trichiura* was at 46%. No polymorphisms at either codon 198 or 200 were detected among the isolated parasite eggs from this study area.

**Conclusion:** It is recommended that periodic molecular screening for drug resistance developing in target parasites be included in control programmes where MDA with albendazole is used.
Influence of nutritional status on pro and anti inflammatory cytokine balance in Plasmodium falciparum malaria children in Imo State, Nigeria

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**Background:** In malaria endemic regions, malnutrition has also been reported to be a public health problem and an important factor modulating the risk of malaria. Pro-inflammatory cytokines are known mediators of malnutrition with higher risk in sub Saharan African Countries. Given the fact that the pattern of the host innate immunity mediated by pro-inflammatory cytokines and the balance between the pro and anti-inflammatory cytokines is critical in determining malaria outcomes. Understanding the impact of malnutrition on pro and anti-inflammatory cytokine response in Plasmodium falciparum (p.f) infected children is very important for malaria control. No Studies in Nigeria has examined the relationship between nutritional status, pro and anti inflammatory cytokine ratio and malaria. We specifically examined the balance between Interleukin 10, Tumour Necrosis Factor (TNF) and nutritional status of plasmodium falciparum malaria children in South Eastern Nigeria. This study determined nutritional status and evaluated the influence of malnutrition on immune response.

**Methods & Materials:** 1344 children aged 1-72 Months with ongoing fever or history of fever within the last 24 hours and with no sign suggestive of severe malaria were microscopically screened for P.f in a cross sectional descriptive study at Ogwa General hospital and Federal Medical Center Owerri, Imo State Nigeria. Their Nutritional status was determined using the international Reference Population defined by the U.S National Center for Health Statistics (NCHS). Blood films were stained with Geimsa stain and malaria identified microscopically. IL-10 and TNF were assayed by ELISA. Statistical analysis was done using SPSS version 17. Study protocol was approved by FMC Owerri ethical committee.

**Results:** Non significant proportions were stunted (22.9%), Under weight (9.4%) and wasted (5.2%). These findings show no significant relationship between presence of malaria and stunting, a measure of chronic under nutrition. Underweight and wasting had no significant relationship with Malaria rather stunting and wasting were associated with age (p< 0.05). IL-10 / TNF ratio was significantly associated with parasite density and age (p<0.05). IL-10 / TNF ratio was lower in children 1-24 months when compared with older children.

**Conclusion:** This study suggests no association between malnutrition and malaria.
Studies on the current status of malaria and its management practices in rural communities of southeast Nigeria

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Background: The effects of malaria are noticeable in rural areas where malaria frequently strikes during that period of the year when the need for agricultural work is greatest. In these areas, the health infrastructure is not sufficiently developed to ensure that an evolving favorable epidemiological situation is maintained. This study was undertaken:
- To assess the prevalence of malaria in different major locations of Onicha-Igbeze community and the practices adopted by the people in the management of malaria.

Methods & Materials: Thick blood films of 100 individuals in the community were used to determine the prevalence. A close ended questionnaire was also administered to 100 respondents in order to obtain information on malaria management practices of the people.

Results: The prevalence of malaria at major locations of the community was as follows: Amanator Primary School (68.2%), Onicha General Hospital (56.3%) and Afoudo market, Onicha (66.7%). The prevalence by age was found to be 1-10 (76.5%), 11-20 (54.5%), 21-30 (50%), 31-40 (57.1%), 41-50 (100%), 51 and above (66.7%). The prevalence by occupation was as follows: students (70%), civil servants (30%), Farmers (72%), and Traders (53.3%). Gender-wise, the males had a total prevalence of 67.9% while females had a total prevalence of 59.1%. The malaria management practices showed that 14% buy antimalarial drugs across the counter, 8% attends hospitals, 14% use traditional medicine from local healers. It was found that some individuals use more than one method in their management of malaria. Those who combined antimalarial drugs from shops with attendance to hospitals were found to be 16%, 12% combined antimalarial drugs from shops with traditional medicine from local healers, 10% combine attendance to hospitals and use of traditional medicine from local healers, 12% do the three management practices, while 14% reported doing nothing about malaria.

Conclusion: The fact that a good number of people buy antimalarials from shops calls for stronger commitment by various authorities to ensure that only genuine drugs are on our counters, and the observation that a good number of people use only traditional medicine from local healers to manage malaria calls for an in-depth basic and strategic research on this line of malaria management.
Moringa oleifera leaf powder role in reinfection pattern of soil-transmitted helminth infection amongst children in Nigeria

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**Background:** There have been several efforts at controlling soil-transmitted helminth (STH) infection with deworming programmes implemented yet, the prevalence and reinfection rates are still high among poor and rural communities. Continuous exposure to the source of infection remains the plausible explanation to the high reinfection rates. Several studies done in rural areas showed that STH reinfection can occur as early as 2 months after complete deworming (Norhayati et al., 1997, Luoba et al., 2005). It has also been reported that hookworm reinfection can occur soon after treatment (Haswel-Elkins et al., 1988).

**Methods & Materials:** A total of 420 (214 male and 206 female) primary school pupils who consented and assented orally to the study protocol after obtaining ethical approval from Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi. The study aimed at evaluating the use of Moringa oleifera leaf powder (MOLP) in STH reinfection interval among school children in south east Nigeria. A randomized, longitudinal, double-blind, placebo control trial with multiple follow-ups using MOLP with Modified Standardized Jollof Rice (MSJR) as treatment while MSJR as control, all served as mid-day lunch packs for 10 months. All the participants were dewormed with 400mg of Albendazole at the baseline before exposure.

**Results:** Data obtained was analysed using SPSS version 20 while proportion was used for descriptive data such as prevalence of STH. The overall STH infection prevalence rate using Kato Katz technique was 120 (28.6%), with 63 (52.5%) females and 57 (47.5%) males infected at baseline. The prevalence of Ascaris lumbricoides was 61.7%, hookworm 30% while Trichuris trichiura was 8.3%. Stool analysis at the 2nd and 6th month recorded no STH infection in both the treatment and control groups. However, at the 10th month, overall prevalence reduced to 63 (15%), with 36 (30%) females and 27 (22.5%) males infected. Also, the post-treatment prevalence for A. lumbricoides was 14.2%, hookworm 0.3% while T. trichiura was 14.2%. Most reinfected children expelled the larvae. Interestingly, treatment groups (11.7%) recorded a marked reduction in STH reinfection compared with the control (40.8%).

**Conclusion:** MOLP has the potential of expelling the adult larvae of STH infected children with prolongation of the reinfection interval.
Common parasites prevalent among school children in Nnobi, Idemili South Local Government Area, Anambra State, Nigeria.

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Background: The distribution of parasitic diseases in any community is usually wide spread but uneven. These infections are malaria, helminthes or arthropods and their prevalence among the various population segments is said to be tilted heavily towards women and children of school age due to obvious reasons: higher exposure to infective agents. Many surveys abound as to the situation in many communities in various parts of Nigeria. This paper presents a study conducted in Nnobi, a major town in Idemili South Local Government Area of Anambra state, using zinc sulphate flotation method and microscopy to determine and expose the parasites prevalent among school children in the area.

Methods & Materials: The study population was 200 school-aged children of Nnobi town including, 105 males and 95 females, aged within 5-20 years. A total of 200 stool samples used, were collected into sterile sample bottles, each bottle contained 5g of morning stool in few drops of formalin/ethanol as preservatives. Stool samples were processed using Zinc-sulphate flotation method and microscopy. Data on health/hygiene habits like hand washing, shoe wearing and swimming habit, presence of latrine and its usage, water sources for domestic use and other risk factors around were also collected. Other information such as sex, age of children, and occupation of parents were also recorded and data analyzed using frequency distribution table.

Results: Out of the 200 stool samples examined, 85 (42.5%) were infected with Hookworm; 53(26.5%) had S. stercoralis; 21(10.5%) had A. lumbricoides and 29(14.5%) children had both A. lumbricoides and T. trichiuria (mix infection). The prevalence of Girdia. Intestinales 7(3.5%) and Entaemoba histolytica 5(2.5%) were statistically not significant (P>0.05).

Conclusion: The results of the study deduced that parasitic helminthes are prevalent in the study area. Therefore, public health intervention programme should be adopted to help in the control and eradication of this problem.
Diagnosis and treatment of uncomplicated malaria in children at a tertiary hospital in Abuja

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Background: Malaria poses a global threat and challenge to healthcare despite the fact that it is treatable and preventable. Though there are documented evidences on the consequences of misdiagnosis of malaria, presumptive diagnosis is still being widely practiced by clinicians.

Methods & Materials: A retrospective study involving case notes of children who were treated for uncomplicated malaria at a tertiary hospital was carried out. Data on demographics, diagnostic procedures, drugs prescribed, presenting symptoms and clinic revisits within 2 weeks were collected from the case notes. Data was analyzed using descriptive analysis method.

Results: The study showed that of all the 269 cases reviewed, more males 157 [58.4% (95% CI = 58.3% - 58.5%)] suffered uncomplicated malaria than females 112 [41.6% (95% CI = 41.54% - 41.66%)]. The mean age and weight was 32.22 months and 14.23 kg with a standard deviation of ± 29.27 and ± 8.18 respectively. Only 9 [3.3% (95% CI = 3.24% - 3.36%)] children were treated based on positive microscopy results obtained on the basis of emergency few hours after presentation to the clinic. Fifty-two [19.3% (95% CI = 19.24% – 19.36%)] were treated without collecting microscopy results that were requested for while 208 [77.3% (95% CI = 77.24% – 77.36%)] were treated based on presumptive diagnosis. None of the patients with slide negative results were treated for malaria. In addition to the antimalarial drugs prescribed, 147 [54.6% (95% CI = 54.54% – 54.66%)] received antibiotics prescription. The study also revealed that cough was the commonest symptom presented by 142 [52.8% (95% CI = 52.74% - 52.86%)] children followed by gastrointestinal symptoms by 104 [38.7% (95% CI = 38.64% - 38.76%)] of the children. It was also observed that 51 [19% (95% CI = 19.06% - 18.94%)] children revisited the clinic within 2 weeks for review.

Conclusion: Presumptive diagnosis was the major practice employed in the treatment of malaria in this study. This has led to misdiagnosis and overtreatment of malaria. Using faster diagnostic tools like Rapid Diagnostic Test will help resolve these problems.
Giardia lamblia infection in institutionalized Romanian children
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Background: Parasitic diseases have a worldwide distribution and represent an important public health problem. The aim of the present study was to assess the prevalence of intestinal parasitic infections in a Romanian Children Care Unit.

Methods & Materials: One hundred twelve institutionalized children aged 2-6 years were investigated. Stool examinations were performed using the iodine staining for the identification of protozoan cysts and the Willis-Hung concentration method for the identification of helminth eggs. Clinical and laboratory investigations were also conducted to evaluate symptoms and eosinophil count in the infected children. Eosinophil values were determined in the peripheral blood by differential white blood cell count with May-Grunwald-Giemsa staining. The control group consisted of 34 healthy children age matched.

Results: Intestinal parasitic infections were diagnosed in 27 cases (24.1%). Giardia lamblia (19.6%), Entamoeba coli (5.3%), Hymenolepis nana (3.5%), Ascaris lumbricoides (1.7%) and Trichuris trichiura (1.7%) were the only parasites identified. Giardia lamblia was diagnosed in 22 (81.5%) of 27 infected children. Among the children with parasitic infections we have determined association of two parasites in 7 (18.5%). Diarrhea (40.9%), weight loss (45.4%), nervous disorders (31.8%), and cutaneous manifestations (18.1%) were the most frequent clinical signs in children with giardiasis. We have found that in children with giardiasis the eosinophil number was increased (6.34% +/- 4.21%) compared to controls (2.89 +/- 1.38%) (p<0.01). Laboratory results revealed that 59% of the children with giardiasis had the eosinophil count in normal range (≤4%).

Conclusion: In conclusion, parasitic diseases have been diagnosed in a significant number of institutionalized children. Giardia lamblia was the parasite identified most frequently in this Pediatric Care Unit.
The in vivo assessment of antiplasmodial activities of leaves and stem bark extracts of Mangifera indica (linn) and Cola nitida (linn)

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Background: Malaria is a serious parasitic disease from tropical regions caused by species of Plasmodium and transmitted by Anopheles mosquitoes. It is prevalent in countries in Africa, Southeast Asia and South America. High mortality rate is reported in these regions. The exponential increment of resistance of the most severe and commonest form of Plasmodium species, Plasmodium falciparum to chloroquine, a prominent anti malarial drug and first line drug over the past two decades has necessitated the investigation into tradinationally calmed anti malarial plants. Amongst the commonly used anti malarial plants in Nigeria are Mangifera indica and Cola nitida.

Methods & Materials: The air dried leaves and bark of Mangifera indica and Cola nitida were powdered and extracted using aqueous and ethanol as solvents. The solvents along with extracts were drained out and filtered. The semisolid extracts were obtained in vacuum using rotary evaporator. The malaria screened, twenty seven albino mice of both sex with body weight range of 18 to 25g were obtained from Obafemi Awolowo university, Ile Ife, Osun State, Nigeria and were allowed to acclimatize for one week. Each of the Swiss albino mice was intraperitonally administered with 0.2ml Plasmodium berghei parastized red blood cells and the parasitemia level were monitored for five days. After the establishment of infection, the extracts, chloroquine and artesunate(used as positive control were administered orally through intra gastric route using the stomach tube to ensure the safe ingestion of the treatment doses to the tested groups daily for four subsequent days.

Results: The fourth day suppression results revealed a significant reduction in the parasitemia level of the different treatment groups. A percentage suppression of 97.05 % was recorded in the mice group treated with ethanolic Cola nitida leaves extract while 95.82% was recorded in the group treated with ethanolic Mangifera indica leaves extract.

Conclusion: The high suppressive values obtained justified the local usage of the plants as anti malarial plants and can be employed as a potential extracts for the development of active novel drugs against malaria parasites.
Comparison of the incidence of Dientamoeba fragilis in a cohort of paediatric children with allergic asthma and controls: Is it a pathogen or protector?

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Background: Dientamoeba fragilis is a neglected intestinal protist associated with clinical outcomes in humans. There is still an obscurity about its pathogenicity in humans, including whether it can modulate immune responses, through which mechanisms it initiates infection, or it is mostly a silent resident of human gut. There are many studies and case reports which all indicate D. fragilis as the causative agent of patients with diarrhea, abdominal pain, meteorism and urticaria. Yet, D. fragilis may have pathogenic subtypes and/or act as a pathogen under certain circumstances through interaction with the host’s immune system. It was initially identified by conventional methods. After PCR became available, it was identified surprisingly common in regions where parasitosis is uncommon, such as Denmark and the Netherlands. Indeed, results of many controlled studies showed higher D. fragilis presence in healthy controls compared to study groups, which suggested that D. fragilis may be a natural resident of human gut, rather than being a pathogen. Combining these data with current debates about the influence of gut microbiota on human health, we aimed to compare the incidence of D. fragilis in a cohort of children with allergic asthma and healthy controls in this preliminary study.

Methods & Materials: A total of 50 children who were diagnosed as allergic asthma and 46 age-matched healthy controls were enrolled in the study. Personal information was collected from all participants initially. The mean age of asthmatic children was 5.67±2.1 and that of healthy controls was 5.43±3.2. Fresh stool samples were collected, their DNA content was isolated, and Real-Time PCR that targeted the ITS-1 region of D. fragilis was conducted.

Results: The results of the assessments showed that 26 of 50 (52.0%) patients and 36 of 46 (78.3%) healthy controls were positive for D. fragilis. The difference between the groups was significant (p=0.007; OR: 0.301).

Conclusion: Our findings contribute to recent reports in which D. fragilis was found more common in healthy controls. We now plan to assess the role of D. fragilis in immune modulation and its interactions with human microbiota in a larger scale.
Detection and subtype identification of Blastocystis in a hospital setting from southeastern India

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Background: Blastocystis was identified almost 100 years back but its clinical significance is controversial. An estimate suggests that Blastocystis inhabit large intestine of more than 1 billion human worldwide. Based on the ribosomal lineages different species of Blastocystis are designated as various subtypes (ST) with extensive inter and intra subtype genetic diversity. Due to its polymorphic nature identification by microscopy is obscure. However, in India, data pertaining to Blastocystis were chiefly derived from direct stool microscopy. In this study we employed microscopy, culture and PCR for the detection of Blastocystis from stool samples. Further, subtyping of representative samples were carried out to identify the subtypes available in this region.

Methods & Materials: It is a cross sectional analytical study approved by JIPMER Institute Ethics Committee. All the stool samples were screened by routine microscopic investigations and they were subjected to in vitro propagation in Jones' medium. Fecal DNA was extracted by using QIAamp DNA stool mini kit (Qiagen, Germany) following manufacturer's instructions and stored at -20°C. Further, extracted DNA was quantified and subjected to PCR, which targets initial 600 bp barcoding region of 18SSU rDNA of Blastocystis. PCR products were visualized on 1.5% agarose gel and representative positive amplicons were sequenced for subtype analysis. Sequence results obtained from both the strands were assembled and subtype analysis was performed by using following database http://www.pubmlst.org/blastocystis/

Results: A total of 173 stool samples were screened for Blastocystis. PCR detected the maximum number of Blastocystis (n=77, 44%) followed by culture (n=48, 28%) and Microscopy (n=25, 14%). The Sequencing results of the representative PCR amplicons confirmed the presence of Blastocystis ST3 allele 34 (n=9) and ST1 allele 4 in (n=5).

Conclusion: In comparison with stool microscopy and culture, Blastocystis specific PCR is an excellent diagnostic tool in terms of sensitivity, specificity and subtype identification. However, in resource poor settings Jones’ medium (xenic culture) could be used as an alternative diagnostic modality for the detection of Blastocystis from stool. Subtyping results indicate ST3 predominance. However, large number of samples needs to be subtyped to reveal the association of particular ST with particular clinical manifestations.
MicroRNA mediated immune regulation of T helper cell differentiation and plasticity during visceral leishmaniasis infection: A computational approach

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Background: Visceral leishmaniasis (VL) is a tropical neglected disease caused by Leishmania donovani, results in significant mortality in Indian subcontinent. The protective immune response to Leishmania parasites is mediated by proliferation and differentiation of IFN-γ secreting CD4⁺ T helper (Th1) cells while IL-4 dependent CD4⁺ T helper (Th2) cell leads to aggravate VL pathogenesis. The plasticity of T cell proliferation and differentiation depends on microRNA mediated gene regulation which leads Th1/Th2 or Th17/Treg type of immune response during human VL.

Methods & Materials: MicroRNAs participates in T cell proliferation and differentiation in human VL. This study depicts the identification of target immune signaling molecule and transcription factors, which play role in T-cell proliferation and differentiation followed by the identification of miRNA controlling their gene expression using three web servers viz., TargetScan, mirPath and miRDB.

Results: The present study provides the *in silico* evidences that seed region present in the microRNAs miR-29-a, miR-29b and miR29c have the putative binding site in the 3'-UTR region of TBX21 transcription factor of CD4⁺ T helper (Th1), which may suppress the Th1 specific protective immune response. Development of Th2 type specific immune response can be suppressed by binding of miR-135 microRNA over the 3'-UTR region of GATA-3 transcription factor of Th2 specific CD4⁺ T helper cells. Interestingly, miR-21 and miR-24 can inhibit the Th1 immune response and simultaneously activate the Th2 immune response by stimulating T helper cell proliferation and differentiation. We are indicating that miR-135, miR-155 and miR-1272 and miR-223 suppress Th2 specific immune response and maintain the plasticity by activating Th1 specific CD4⁺ T helper cells.

Conclusion: This study indicates that microRNAs have capacity to regulate immune signaling, cytokine production and immune cell migration to control the VL infection in human. This observation warrants further investigation for the development of microRNA based therapy controlling T cell differentiation in human VL.
The epidemiologic considerations about visceral leishmaniasis in Albania 2010-2014
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**Background:** The aim of this study was to analyze some epidemiologic features of visceral Leishmaniasis in Albanian children.

**Methods & Materials:** There were included 194 children aged 0-14 years in this study, all admitted and treated for visceral Leishmaniasis since 1010-2014 in Pediatric Infectious Disease Service. We studied the distribution of the disease according to annual incidence, age, gender, living area.

**Results:** The results are shown in the following table.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>NEW CASES</th>
<th>GENDER</th>
<th>LIVING AREA</th>
<th>AGE (in years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>Rural</td>
<td>Urban</td>
</tr>
<tr>
<td>2010</td>
<td>46</td>
<td>25(54%)</td>
<td>10(22%)</td>
<td>36(78%)</td>
</tr>
<tr>
<td>2011</td>
<td>33</td>
<td>14(42%)</td>
<td>4(12%)</td>
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<td>17(36%)</td>
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<td>36(77%)</td>
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<td>2013</td>
<td>39</td>
<td>17(44%)</td>
<td>22(56%)</td>
<td>25(64%)</td>
</tr>
<tr>
<td>2014</td>
<td>29</td>
<td>11(38%)</td>
<td>18(62%)</td>
<td>23(79%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>194</td>
<td>84</td>
<td>110</td>
<td>149</td>
</tr>
</tbody>
</table>

**Conclusion:** Visceral Leishmaniasis is a frequent disease in Albania presented with a considerable number of cases per year. The most affected age group is from 1-4 years old, the male gender is the most affected and urban areas are also predominant over rural ones.
Cervical cytology as a diagnostic tool for genital schistosomiasis and cervical squamous cell atypia among young women from schistosoma and HIV endemic populations in South Africa


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Background: Globally, Africa has the highest prevalence of HIV, schistosomiasis and another neglected entity: cervical cancer. Genital schistosomiasis manifests in lesions that are hypothesized to link with HIV and cervical atypia.

Methods & Materials: This study was conducted among 833 young women aged 16-23 years from rural high schools in KwaZulu-Natal. Risk factors for schistosomiasis and cervical atypia and the association of genital schistosomiasis and squamous atypia of the cervix were investigated using diagnostic cytology, urine microscopy, and questionnaire data.

Results: Participants were sexually active from a young age and 523 (63.0%) had at least one child and 742 (89.1%) relied on rivers as their primary water source. The Schistosoma prevalence detected cytologically and via urine microscopy was 12 (1.4%) and 178 (21.4%) respectively. Squamous cell atypia was detected among 567 (68%). There was a significant association between the participants who were positive for any squamous cell atypia and those who had S. haematobium eggs in Pap smears (OR= 5.6, P =0.005; 95% CI 1.6 -21.0) and for S. haematobium eggs in urine OR= 2.9, 95% CI 1.72 - 4.99, P=0.005).

Conclusion: The specificity of cytology for Schistosoma detection was low using is seen previously, it is possible that an improved detection rate of genital schistosomiasis could be achieved using cervico-vaginal lavage Schistosoma DNA testing. While a significant association exists between urogenital schistosomiasis as detected by cytology and urine microscopy with squamous atypia in this young population, it must be noted that more than half of the young women have cervical atypia that could potentially regress. Cytology was useful in revealing the squamous atypia among this young population who is not routinely screened, a limitation is that it was not feasible to confirm results using histology or other complementary tests. The relationship between schistosomiasis and cervical cancer is complex, while there may be association, it was not possible to prove causality or eliminate all confounders. In communities at risk, health promotion, screening and health care targeting not only HIV and schistosomiasis, but also cervical cancer should be made available in order to reduce the prevalence of these preventable diseases.
Efficacy of percutaneous interventions in patients with hepatic echinococcosis
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Background: The aim of study was to clarify the biochemical mechanisms of severe hemorrhagic forms of erysipelas and find the possible early predictors of its development.

Methods & Materials: A puncture in patients with hepatic echinococcosis was performed using ultrasonic control. Percutaneous drainage was performed under fluoroscopic control. Protoscolicidal processing and cyst shells degradation were performed with 1% aqueous solution of hypochlorous solution. After percutaneous intervention the patients had received conducted anti-relapse therapy with albendazole (800 mg/day, 3 courses within 28 days with an interval of 14 days).

Results: During 2004-2015 transdermal interventions were performed in 58 patients (37 women, 21 men in the age of 53 +/- 4 years) with liver cysts. Single cysts were in 30 (51.7%) patients, multiple in 28 (48.3%). The maximum number of cysts in one patient was 10. According to the ultrasonic and CT maximum longitudinal size of the cysts ranged from 9 mm to 167 mm. Totally it was performed 91 percutaneous interventions, 27 punctures, 64 drainages.

44 percutaneous intervention were performed for cysts type I (according to the classification of Gharbi), 6 – for type II, 28 – for type III, 5 – for type IV, 8 – for type V. The effectiveness of percutaneous treatment was 98.2% (57 patients). Intraoperative complications (allergic-type urticaria) were observed in 2 (3.4%) patients. Surgical complications in the postoperative period were recorded in 16 patients (28.1%) patients: external biliary fistula in 9 (15.8%), bleeding cavity of cyst in 1 (1.8%), suppuration of the residual cavity in 1 (1.8%), bile-bronchial fistula in 1 (1.8%). 4 (7%) patients had external biliary fistula in combination with suppuration of the residual cavity in 2 (3.5%) and bleeding cyst in 2 (3.5%). Complications have been cropped with conservative therapy. Relapse was observed in 1 (1.7%) patients who did not receive anti-relapse therapy.

Conclusion: The results showed high effectiveness of echinococcosis treatment with percutaneous interventions and conservative therapy with albendazole and using as protoscolicidal preparation 1% sodium hypochlorite.
Features of dirofilariasis in the Northwest of the Russian Federation
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Background: Dirofilariasis is the only vector-borne worm infection in countries with a temperate climate. Novgorod Region is the northern border of dirofilariasis habitat in Russia. The first indigenous case of dirofilariasis was registered in 2011 in Novgorod Region.

Methods & Materials: Study was conducted in the period from November 2010 to July 2015. We investigated 235 samples of dog blood. Samples assay was conducted by concentration method with 3% acetic acid. We studied 438 female mosquitoes.

Results: In medical network of Novgorog region 11 cases of infestation of humans by Dirofilaria repens were observed during this period. In all cases parasites were identified as immature females with 120-140 mm of body length and its width of body was 0.4 to 0.5 mm. The infestation by dirofilariasis was identified in 59 (25,1%) dogs. Of 235 surveyed animals 81 were the service dogs belonged to the Ministry of Internal Affairs. We revealed a high infestation of the service dogs by Dirofilaria repens (34,6 %). The course of anthelmintic drugs was administered to service dogs. During the follow-up period of one month after treatment a 5.1% decrease of the infestation by dirofilariasis has been found in the service dogs. During the observation period infection of female mosquitoes was decreased from 0,9% in 2011 to 0,3% in 2014.

Conclusion: Studies have shown that in the Novgorod region adoptable conditions are present for formation of the foci of dirofilariasis. Detection of human dirofilariasis in Northwest of Russia has been associated with delivery of infected service dogs of Ministry of Internal Affairs. Service dogs are regularly seconded to work in the heartworm-endemic territories regions like as North Caucasus or Southern Russia. In our view, the circulation of Dirofilaria repens in the Novgorod region supports a high number of infected dogs and a lot of vectors of transmission. Abnormally warm summers of 2010 and 2011 contributed to the development of several generations of larvae vector transmission. These factors could lead to the forming the foci dirofilariasis in the Novgorod region and the emergence of autochthonous cases of Dirofilaria repens invasion in humans.
Antibodies to plasmodium falciparum glutamic acid rich protein (PfGARP) inhibit parasite growth by arresting trophozoite development

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Background: Malaria affects almost one-half of the world’s population and causes more than 600,000 deaths annually. Young children in malaria endemic areas of Africa have the highest mortality rate because of their immature immune systems. In previous vaccine discovery efforts, we developed a differential screening method using plasma from children who were resistant or susceptible to falciparum malaria. Using this approach, we discovered PSEA-1. Antibodies to PSEA-1 predict resistance to severe disease in two yr old children, block schizont egress from infected RBC in vitro, and vaccination with rPbSEA-1A protects mice from P. berghei ANKA challenge (Raj et. al Science 2014).

Methods & Materials: We have now adapted our differential screening method to field parasite-derived phage display libraries. In differential bio-panning assays, PfGARP (aa 410-673) was recognized by plasma pooled from resistant (n=11) but not susceptible (n=14) children participating in our birth cohort in Muheza, Tanzania. To further characterize PfGARP, we generated mouse antibodies against this immuno-relevant, highly invariant region (PfGARP-A) and performed growth inhibition (GIA) and immunolocalization studies. For GIA, 3D7 parasites were synchronized to the ring stage and plated at 0.3-0.4% parasitemia in the presence of anti-PfGARP-A or pre-immune sera (1:10 dilution). Parasites were cultured for 48 hrs and ring and early trophozoite stage parasites were enumerated.

Results: Anti-PfGARP-A inhibited parasite growth by 99% compared to controls (P < 0.001). In confocal studies, PfGARP localized to the RBC membrane in trophozoite and early schizont infected RBCs, but not to other parasite stages or uninfected RBC. To determine the mechanism of growth inhibition we performed trophozoite arrest assays (TAA) using anti-PfGARP-A. For TAA, 3D7 parasites were synchronized to the ring stage and plated at 5% parasitemia in the presence of anti-PfGARP-A or pre-immune mouse sera (1:10 dilution). Parasites were cultured for 36 hrs and trophozoite stage parasites were enumerated. Anti-PfGARP-A arrested trophozoite progression by 99% compared to controls (P < 0.001)
**Conclusion:** These data support Pf-GARP as a novel vaccine candidate for pediatric *falciparum* malaria. By blocking trophozoite development, PfGARP may synergize with vaccines targeting hepatocyte and red cell invasion and schizont egress.
HLA determinants of susceptibility and protection to L. donovani: in silico analysis

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Background: Sri Lanka is endemic for leishmaniasis caused by Leishmania donovani Mon 37. Localised cutaneous leishmaniasis (LCL) is the predominant presentation with a few reports of visceral disease. Limited differences in the two strains causing cutaneous and visceral phenotypes (1) suggest concomitant operation of host factors. A case-control association study conducted by us suggested HLA*A68 and HLA*B07 to confer protection to LCL (submitted for publication). The objective of this study was to further assess these findings by predicting MHC-I binding for nonameric peptides in identified L. donovani sequences.

Methods & Materials: Protein coding genes of L. donovani reference strain BPK282A1 were retrieved from TryTripDB. Protein sequences of genes (LdBPK_365490.1, LdBPK_303700.1, LdBPK_261610.1 and LdBPK_220670.1) shown to be over expressed in L. donovani strains causing cutaneous and visceral disease in the country were identified. The MHC -I binding predictions for these peptides were made using the IEDB analysis resource Consensus tool. Binding of HLA alleles associated with LCL were compared with the 5 most common alleles at these loci (threshold percentile rank <=1). Common alleles were selected according to data available for the local population.

Results: The total number of epitopes predicted for selected alleles at HLA-A and HLA-B loci were 70 and 52 respectively. At the HLA-A locus, A*68 had the highest number of predicted epitopes (n=19). Majority of these (n=13) were on adaptin protein coded by LdBPK_365490.1 and 11 of them were high affinity binders (IC50 <50 nM). At the HLA-B locus, B*07 was predicted to bind the most number of epitopes (n=14) with a similar distribution in proteins encoded by LdBPK_303700.1 and LdBPK_365490.1. One epitope each on A2 protein was predicted to bind HLA-A*02 and HLA-B*57. No epitopes on this protein were predicted for HLA*A68 and HLA*B07.

Conclusion: Alleles which had an apparent protective effect according to our previous findings consistently demonstrated ability to bind more epitopes, of proteins encoded by genes over expressed in parasite strains causing LCL. The immune responses in this host-parasite combination should be studied in a larger sample, in a HLA restricted setting, to elucidate any protective mechanisms.
Evaluation of loop mediated isothermal amplification for the diagnosis of amoebic liver abscess

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Background: Entamoeba histolytica is an important cause of amoebic liver abscess (ALA) in humans, particularly in developing countries. Molecular techniques are increasingly used for the detection of infectious agents in clinical samples with high sensitivity and specificity. The present study has been planned to investigate the utility of Loop mediated isothermal amplification for the detection of E. histolytica/ E. dispar in suspected cases of amoebic liver abscess.

Methods & Materials: A total of 138 pus samples were collected from the suspected patients of ALA who attended emergency medical OPD/Liver clinic of Postgraduate Institute of Medical Education and Research, Chandigarh / Government Medical College and Hospital, Sector-32 Chandigarh from November 2013 to November 2014. Pus samples of liver abscess were obtained by ultrasound guided aspiration and subjected for diagnostic evaluation using Loop mediated isothermal amplification and Real time PCR for the detection of E. histolytica/ E. dispar. ELISA was performed for the detection of anti-Entamoeba IgG antibodies on the corresponding serum samples.

Results: The M:F ratio of the recruited patients was 16.1:1. Loop mediated isothermal amplification detected E. histolytica DNA among 58 of 133 (43%) pus samples as compared to Real time PCR which detected 28 of 133 (21.66%) pus samples. Anti-Entamoeba antibodies were detected by ELISA in serum of 99% of cases.

Conclusion: Thus, loop mediated isothermal amplification proves to be a promising diagnostic tool in providing direct detection and confirmatory evidence of amoebic liver abscess in comparison to Real time PCR and antibody detection by ELISA.
Hemoglobinuria (Black Water Fever) in severe falciparum malaria – a case report
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Background: Introduction:
Black water fever (BWF) is a syndrome characterized by intravascular hemolysis, acute renal failure and the passage of black urine in severe Plasmodium falciparum infection when treated with amino-alcohol drugs, including quinine, mefloquine and halofantrine. BWF is a rare manifestation of falciparum malaria characterized by sudden intravascular haemolysis followed by fever and haemoglobinuria. We present a rare case of black water fever in Kuwait.

Methods & Materials: Case summary:
A 20 year old Nigerian boy came to Infectious Diseases Hospital (IDH) with high fever (40.8°C), heart rate 125, blood pressure of 100/60 mmHg and physical examination was unremarkable.

Results: Results:
The initial laboratory tests revealed mild anemia (Hb, 120g/L and HTC, 0.352 L/L, RBC, 4.12 10^12/L, WBC, 4.2 10^9/L and platelets 16 10^9/L). His G6PD was normal, 214.6 mU/10^9 erythrocytes, (normal range 165 – 365 mU/10^9). The thick and thin blood examination confirmed the severe infection of Plasmodium falciparum with 41.0% parasitemia. The patient was admitted and started intravenous Quinine. The patient was feeling much better on next morning but became unconscious by evening and shifted to ICU. His all CBC parameters were higher and started passing cola color urine. The 12 units of whole blood was exchanged on 3rd day. He became fully conscious on 4th day. His anemia and thrombocytopenia was improved and the color of the urine also became normal.

Conclusion: Discussion:
The mechanism of BWF is unknown but associated with high density of P. falciparum, partial malaria immunity, G6PD deficiency and treatment with amino-alcohol drugs. The patient had a history of two times of malaria infection and partial immunity are associated with BWF seems to have been his only risk factor.

Conclusion:
Quinine is managing both complicated and uncomplicated malaria and may precipitate black water fever in severe infection of P. falciparum. The black water fever is caused by the hemolysis of erythrocytes due to malaria and also with the metabolism of quinine by the cytochrome P450 3A4 enzyme responsible for increasing oxidative stress within erythrocytes, making these cells more vulnerable to hemolysis with falciparum malaria and/or G6PD deficiency.
Role of B-cells and antibodies in visceral leishmaniasis infection
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Background: The immune mechanism for subverting the parasitic disease depends on both the innate and cell-mediated immune responses. The role of B-cells in the protection towards the disease could be attributed due to its antibody production, antigen presentation and cytokine production events; they may play a protective or pathological role. This study aims to look for the role of B-cells in induction of immune activation, disease associated anemia and ultimately in parasitemia control.

Methods & Materials: The study was performed on whole blood/PBMC and splenic aspirates from VL cases and endemic control group whole blood/PBMC. The aspirates from spleen of patients were obtained for diagnosis and scored upon microscopic examination. Blood from cases and subjected to flow cytometry for assessment of the frequency of B-cell and the costimulatory molecules (CD80 and CD86) along with the activation status (CD25). The measure of humoral responses were assessed through the positive antibody titres by DAT and haemoglobin levels were obtained from the plasma samples of cases.

Results: There was significant difference in the frequency of B cells in cases pre- and post-treatment (p=0.0007) however no significant differences were found between the pre-treatment group and the healthy controls. The activation of B-cell was significantly decreased (p=0.009) in VL cases as compared to the healthy controls. The frequency of CD19+ cells with CD80 expression differed between the heathy controls and the post-treated individuals with p-value=0.0013. CD86 expression was significantly higher (p=0.0001) in B-cell population from post-treated as compared to pre-treatment. The splenic score was in accordance with the antibody titres and the haemoglobin levels in the VL cases.

Conclusion: We found that the B-cells have important role in driving immune responses demonstrated by the increased frequency as the treatment progresses and gradually resumes the normal level upon cure while the activation upon treatment and persists even after cure. The understanding of the role of B-cells in the infection could be a major breakthrough for designing a therapeutic option which could be of help in the clearance of the infection.
Deciphering the interplay between cysteine synthase and thiol cascade proteins in the survival of *L. donovani* under oxidative stress

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**Background:** *Leishmania* possess a unique trypanothione-dependent redox metabolism with pivotal role in protection from oxidative damage and drug resistance. The cascade of trypanothione biosynthesis depends on L-cysteine as the precursor, whereas, cysteine bioavailability is itself dependent on the cysteine biosynthesis pathway which includes enzyme cysteine synthase (CS). However, despite the apparent dependency of redox metabolism on cysteine biosynthesis pathway, the role of CS in drug resistance and redox homeostasis has remained unexplored. Herein, we have attempted to investigate the role of LdCS in Amphotericin B (Amp B) sensitive vs. resistant isolates of *L. donovani*.

**Methods & Materials:** LdCS was cloned in pXG-GFP⁺ vector to express LdCS as fusion proteins with a C-terminal GFP tag. The construct LdCS-GFP was transfected by electroporation in the *L. donovani* sensitive strain promastigotes and transformants selected upto final concentration of 200 µg/ml G418. MTT assay was performed to determine IC₅₀ value of LdCS-GFP overexpressor and Amp B sensitive strains of *L. donovani* under different ROS inducers such as, H₂O₂, menadione and SNAP. Further, ROS levels, thiol content and enzymatic activities of LdCS, peroxidase and SOD were analyzed.

**Results:** Our results demonstrate stage-specific increase of LdCS expression and its enzymatic activity, accompanied by a higher thiol content, which implies that LdCS is upregulated in Amp B resistant isolates and during stationary stages of growth to meet the increased thiol demand of respective stages/isolates culminating into enhanced stress tolerance. In fact, overexpression of LdCS-GFP in sensitive strains imparted enhanced oxidative stress tolerance to the over-expressing parasites as compared to the wild type (WT) parasites. The IC₅₀ values of LdCS-GFP toward H₂O₂, menadione and SNAP was found to be 217 ± 8.7 µM, 16.5 ± 2.5 µM and 370 ± 9.7 µM, respectively, which was ~1.87, ~2.21 and ~1.34 fold higher than WT parasites. Furthermore, enzymatic assays, thiol content and immunoblot analysis showed that these oxidants induced LdCS-GFP, as well as endogenous CS and thiol cascade proteins expression in *L. donovani* suggesting a ROS regulated mechanism of LdCS expression and thiol pathway proteins.

**Conclusion:** The LdCS expression is modulated by ROS probably to cater the metabolic demands of trypanothione and hence, alleviate oxidative stress.
Investigating changes in monocyte phenotypes and functions in active visceral leishmaniasis patients

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Background: Visceral Leishmaniasis (VL) is a major public health problem in the Indian subcontinent. Mononuclear cells have direct role in initiation of inflammation, anti parasitic defences and ultimately in maintenance of tissue homeostasis. Monocytes play an important role as immune effector cells but their role in VL is not well known. We hypothesised that M2 macrophages play an important role in VL pathogenesis and aimed to characterise monocytes phenotypically and functionally.

Methods & Materials: We established M1 and M2 macrophage polarization dynamics in the whole blood of active VL patients at an intervals of 7 days for four weeks until their drug treatment was complete. Monocytes were phenotypically charactereised by immunophenotyping studies for chemokine receptors CCR2, CX3CR1, CCR7 and cell adhesion molecules VCAM1, ICAM1, PECAM1. Functional characterisation includes quantitation of intracellular TNF-α, IL-6, IL-1β production, gene expression level measurement of various M1, M2 markers in CD14 enriched cells from active and cured VL cases and in endemic healthy controls. Myeloperoxidase expression, phagocytosis and intracellular Leishmania parasite killing by monocytes were also examined.

Results: We found only minor changes in the frequency of M1 and M2 macrophages at the beginning and end of drug treatment, but observed several significant changes in the expression of cell surface markers (CD206, CD163) used to identify these cell subsets. In particular, we found that M2 macrophages increased in frequency and changed expression of cell surface molecules 14 days after drug treatment commenced, suggesting a potential role in tissue repair and homeostasis. We also observed significantly upregulated expression of several M2 markers (TGM2, PKM, SLC2A1) at gene level. We found reduced expression of CD14 on monocytes from active VL patients, but exacerbated TNF-α, IL-6 and decreased production of IL-1β in response to SLA/LPS stimulation, compared with the same cells from drug cured and endemic control samples. We also found decreased myeloperoxidase expression by monocytes in VL.

Conclusion: Together, our findings indicate dynamic changes to macrophage and monocytes populations in VL patients over the course of drug treatment, and suggest that the functions of these cells may change at different stages of disease. We found upregulation of some markers for monocyte deactivation.
Microplate whole blood interferon-γ release assay for marker of Leishmania donovani infection

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Background: Laboratory tests which can be produced in a reproducible and scalable manner are much needed to identify visceral leishmaniasis (VL) infection and fulfill the goals of the elimination campaign. Whole blood interferon gamma (IFN-γ) release assay (IGRAs) is an in vitro immune test that has recently been developed as an alternative to the Leishmanin skin test (LST) for identification of individuals exposed to L. donovani infection but without disease. Requirement of 3 ml of blood preclude this test widely acceptable for larger community based studies. This study aimed at evaluating the performance of microplate based IGRA using 300µl blood (direct blood as well as 1:1RPMI diluted blood) with conventional IGRA (3ml blood) to establish this assay as an epidemiological tool for marker of infection.

Methods & Materials: We employed conventional IGRA and microplate based IGRA with direct as well as diluted venous blood using soluble leishmania antigen (SLA) on patients with active visceral leishmaniasis (VL, n=32), patients with cured VL (n=20), endemic Healthy Controls (EHC, n=21) and healthy control subjects living in non-endemic area (NEHC, n=12). IFNγ levels in culture supernatants were measured by ELISA and kappa statistics was used to access the concordance between test assay formats.

Results: The whole blood cells of both active VL and cured VL produced significantly level of IFN-γ in both format of IGRA. Positive correlations were found with active VL blood in IFN-γ production between 3ml vs 600µl, 3 ml vs 300µl, and 600µl vs 300µl, while with cured VL blood it was moderate with 3ml vs 600µl; 3ml vs 300µl. No significant difference in the overall IFN-γ response by both assay formats was detected, and agreement between tests was significant.

Conclusion: We demonstrate a reliable and scalable process similar in sensitivity to conventional IGRA, but with the advantage of 10 times less venous blood requirement and higher through-put. Development of microplate based IGRA format will be useful tool for providing the means to more efficient screening in large scale immunological and epidemiological studies and fill an unmet need in the VL elimination campaign on the Indian sub-continent.
Decreased miltefosine susceptibility in clinical isolates of Leishmania donovani derived from visceral leishmaniasis and post kala-azar dermal leishmaniasis: Apparent mechanisms and clinical implications

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**Background:** Miltefosine is an oral antileishmanial drug. Recent reports indicate a significant decline in its efficacy and high relapse rate in visceral leishmaniasis (VL) and post kala-azar dermal leishmaniasis (PKDL). Miltefosine susceptibility of relapse case isolates were >3 fold lower compared to pre treatment isolates.

**Methods & Materials:** To understand mechanism responsible for miltefosine unresponsiveness, we determined (i) the sequence of LdMT and LdRos genes implicated in miltefosine translocation (ii) miltefosine accumulation and reactive oxygen species (ROS) tolerance and (iii) transcriptome profile in clinical isolates of Leishmania donovani obtained from VL and PKDL patient at pre treatment and clinically relapsed stages as well as miltefosine resistant parasite(LdM30).

**Results:** LdMT gene sequencing revealed the previously reported single-nucleotide polymorphism, C1259→A resulting in substitution of Thr 420→Asn and a novel SNP T527-A resulting in substitution of Val176-Asp resistant parasites. L. donovani parasites from VL and PKD L patients relapsed after miltefosine treatment exhibited significantly lower accumulation of miltefosine compared with wild type parasites. Miltefosine induced ROS levels were significantly low (p<0.05) in macrophages infected with LdM30 and parasites from relapse cases compared to wild type parasites, indicating better tolerance for oxidative stress in unresponsive clinical isolates. Transcriptome profiling revealed that several genes involved in antioxidant defense mechanism (TRYP, Cyt b5 Red, TSH), metabolic process (Lipase precursor, PGMPUT), transporters (VPTM, MDRP), cell component and cell motility (SMP2, NUP155, CYP) are preferentially expressed in LdM30 and relapsed case parasites than wild type L. donovani parasites. Several other genes mainly transporters like ABCF2, amino acid transporter, surface acylated putative protein, APH and mitochondrial precursor peptide, chaperon TCP20, clathrin coated assembly protein, C5 sterol desaturase, autophagy protein ATG10 were preferentially expressed in wild type parasite compared to relapse case and LdM30 parasites.

**Conclusion:** The present study provides the understanding of parasitic factors and pathways responsible for miltefosine unresponsiveness in VL and PKDL. Decreased miltefosine susceptibility and increasing relapse rate in VL and PKDL patients indicate the declining efficacy of monotherapy with miltefosine and warrants the need of introducing alternate drugs/combination therapy with miltefosine.
Immunomodulatory and toxicological safety studies of two novel anti-leishmanial compounds

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Background: Leishmaniasis is a parasitic disease which can manifest in various clinical forms and the visceral form is highly fatal if not treated. The drugs available are either highly costly or have poor efficacy against the parasite. Hence there is a need to find new molecules and compounds for the treatment of this disease. However, to put the new compounds in clinical trials, their toxicity and immune-modulatory effects must be ascertained to avoid harmful effects on the host.

Methods & Materials: Putative immunomodulatory activity of two advanced preclinical anti-kinetoplastid candidates (KIND0001589 and KIND0001591) was evaluated, using primary human peripheral blood cells from healthy controls and kala-azar patients. Amphotericin B was used as standard drug control. The optimal concentrations of KIND0001589 (0.0134 µg/mL) and KIND0001591 (0.03µg/mL) was used, to pre-treat the peripheral blood mononuclear cell, for 14 hours followed by addition of, PMA (25 ng/mL) and Ionomycin (1 µg/mL) for 4 hours together with Brefeldin A (1 µg/mL). Cell viability was assessed by determining the frequency of Annexin V /7AAD positive cells in CD3+, CD14+ and CD19+ leukocyte subpopulations through BD Influx FACS system. Immunophenotyping was determined in CD3+, CD4+ and CD3+, CD8+ T cells, CD14+ monocytes and CD19+ B cells. The frequency of T-cells, monocytes and B-cells expressing IFNγ, and TNF-α, respectively, was determined by the same flow cytometer.

Results: Short term ex-vivo immunophenotyping assay showed KIND001589 induces CD4+TNF-α and CD4+IFNγ including IL-4, IL-10 and IL-17, in all patients. Where as KIND0001591 induced a short term immunostimulation of CD8+ T cells. Both KIND000189 and KIND0001591 highly induced CD14+ monocytes (CD14+IFNγ and CD14+TNF-α) as compared to the standard drug. KIND0001589 does not induce any B cells mediated immuno-stimulation where as KIND0001591 induced immuno-stimulation in one Patient. KIND0001589 showed stronger short term immunomodulatory activity than KIND0001591.

Conclusion: Both the novel compounds (KIND0001589 and KIND0001589) were found to be safe and potential anti-kinetoplastids for animal and human trials.

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Immunophenotyping of whole blood from kala-azar Patient 3
Malaria in Hong Kong: Impact, eradication and legacy
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Background: This presentation explores how the impact of malaria and the process of its eradication shaped the development of Hong Kong, its scientific and medical expertise and legacy for preparedness.

Methods & Materials: Primary sources - surviving colonial medical records, journals, diaries, newspapers and government reports were analysed for quantitative and qualitative information.
Secondary sources - peer-reviewed journal articles, books and online sources (webpages, libraries, search engines).

Results: Malaria had a major impact on the development of Hong Kong. High disease mortality in the early years of the colony influenced where and how people lived, as well as driving local medical expertise. Initially, the cause was attributed to Hong Kong’s insalubrious environment and miasmatic presumptions dominated Western and Chinese medical thinking. In the absence of scientific delineation, fever-inducing diseases including malaria were typically grouped together, which led to a local catchall moniker ‘Hong Kong Fever’. Discoveries of the etiology and transmission of malaria proved key to the development of effective control methods and as Hong Kong grew, targeted public health measures became increasingly important. The colonial government’s determination to build a viable long-term colony in a widely malarious region necessitated local problem solving. The establishment of a dedicated ‘Malaria Bureau’ in 1930 provided the targeted approach necessary to provide a holistic solution to the problem.

A number of challenges threatened progress against the disease, including waves of migrants from neighbouring malarious countries (in particular mainland China) and the disruption of the Second World War. Eradication of indigenous cases was eventually realized in 1969, nearly 130 years after Hong Kong was colonised. Periodic outbreaks continued to occur as late as the 1980’s fuelled by residential development of the New Territories, the influx of Vietnamese refugees and imported immigrant and tourist cases, threatening a reintroduction of malaria in local mosquito populations.

Conclusion: The eradication of malaria in Hong Kong was a gradual process dependent on scientific knowledge, medical expertise, colonial government policy, public education and vigilance. As an emerging disease ‘hotspot’, these factors are particularly relevant in twenty-first century Hong Kong and remain inherent features of local infectious disease preparedness planning.
Correlation between albendazolesulphoxide in plasma and hydatid cyst and clinical outcome in patients with liver echinococcosis

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**Background:** To investigate the relationship between plasma and cyst concentrations of albendazolesulphoxide (ASO) and their effects on parasitological findings and disease recurrence in patients with liver hydatidosis.

**Methods & Materials:** The study was conducted at the University Hospital for Infectious Diseases “Dr. Fran Mihaljević,” Zagreb, Croatia, between August 2006 and January 2011. Consecutive patients (N = 48, age 6-77 years) were treated with albendazole (3 × 5 mg/kg/d) over 28 days before surgical cyst removal (n = 34) or percutaneous evacuation (PAIR) (n = 14). Plasma ASO was determined on days 10 and 28 of treatment and cyst concentrations at surgery/PAIR.

**Results:** Disease recurred in 3 surgically treated patients. Variability of ASO concentrations was substantial. Plasma concentrations on day 10 were higher than on day 28 (geometric means ratio [GMR] 2.00; 95%CI 1.38-2.91, P < 0.001) and higher than cyst concentrations at the time of treatment (GMR = 1.58, 1.01-2.34, P = 0.045). Higher cyst (but not plasma) concentrations were independently associated with lower odds of protoscolex motility (OR = 0.23, 0.01-0.70, P < 0.001) and higher odds of protoscolex destruction (OR = 1.17, 1.04-1.46, P < 0.001). With adjustment for age and protoscolex motility, higher day 10 plasma concentrations (but not cyst concentrations) were associated with lower odds of disease recurrence (OR = 0.49, 0.09-0.97, P = 0.035). Plasma concentrations did not predict cyst concentrations.

**Conclusion:** Viability of protoscolices progressively decreased with increasing ASO concentrations in the cyst. Data strongly suggested that higher plasma concentrations reduced the risk of disease recurrence.
Comparative analysis and identification of immunoreactive and dominant proteins of cysticercus cellulosae antigens by 2D-Electrophoresis and MALDI-TOF

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Background: Neurocysticercosis (NCC) a disease, caused mainly by inadvertent lodging of the oncospheres of *Cysticercus cellulosae* in the central nervous system (CNS). The pleomorphic nature of clinical manifestations in NCC is based on the presentation of the cyst infestation, which differs with individuals. This hinders the early diagnosis of NCC and demands a specific diagnostic target. 2D electrophoresis combined with MALDI-TOF serves as a tool, aiding our purpose.

Methods & Materials: The different native antigen preparations like, whole cyst, cyst fluid, cyst wall with scolex and excretory secretory antigen from oncospheres of *Cysticercus cellulosae* was carried out using the conventional method of sonication. The proteins were purified (Ready-Prep 2D cleanup kit - BioRad) and standard concentration of protein sample was subjected to 1D isoelectric focusing in 7cm/17cm strips of 3-10 and 4-7 pH ranges, in a linear gradient with an overnight rehydration (Protean IEF Cell, Biorad). And the second dimension was run in conventional 10% SDS-PAGE gels. The gels were processed in two ways, semi-dry blotting and staining to visualize spots. Blotted membranes were treated with positive and negative controls for human NCC and the immunoreactive spots were identified. The dominant and immunoreactive spots were characterized by MALDI – TOF.

Results: SDS-PAGE silver staining showed similar banding profile with varying intensities among the different antigens but second dimensional electrophoresis displayed different spots. Immunoreactive spots after EITB and dominant spots 27-44 kDa, in the 4-5 pH range were trypsin in-gel digested, and molecular weight determination for the spots were carried out. Subsequently followed by peptide mass fingerprinting and MALDI-MS analysis.

Conclusion: This identification and preliminary characterization of the native proteins is useful in selecting a novel and specific protein target, which can be a helpful in accurate diagnosis of NCC and can also serve as a vaccine candidate.
The possible roles of IPT and ITNS in gestational, placental and cord blood malaria parasitemia, pregnancy outcome and fetal weight in Isu, Imo State Nigeria  

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**Background:** Pregnant women in varying stages of pregnancy consented to participate in this study aimed at assessing the possible roles of the use of intermittent preventive treatment (IPT), insecticide treated bed nets (ITNs) in addition to the routine prophylactic malaria drug (Paludrine) on gestational, placental, cord blood malaria parasitemia, pregnancy outcome and weight of the new born.

**Methods & Materials:** Ethical considerations and approvals were duly obtained. Pregnant women were placed in 3 groups: Group A had women who were given IPT in addition to the routine Paludrine, Group B had women given ITN in addition to Paludrine while Group C had women who were given both IPT and ITNs in addition to the routine Paludrine. Peripheral malaria parasitemia was determined from Giemsa stained thick and thin blood smears on the first day of this study and on the day of delivery. Placental and cord blood parasitemia were determined from blood taken from the maternal side of the placenta as well as from the cord of the new born babies. Pregnancy outcome was noted for each participant and all the babies born were weighed on delivery.

**Results:** An initial overall 47.4% peripheral malaria parasitemia was observed. Group A women: 25% had peripheral, 54% Placental and 53% cord blood malaria parasitemia respectively with pregnancy outcome as follows; 13% abortions, 8% still births and 79% live births. The baby birth weights were 12.6% low births, and 12.6% above 4kg. Group B women: 21% had peripheral, 34% placental and 38% cord blood parasitemia respectively with pregnancy outcome as follows: 8% abortion, 5% still births and 87% live births. The baby weights were: 7.6% low birth weights, and 26.1% above 4kg. Group C: 2.3% of the women had peripheral parasitemia, 44.2% had placental and cord blood malaria parasitemia respectively with pregnancy outcome as follows: 3.1% still birth and 96.9% live births. Low birth babies were not observed and 36.4% weighed above 4kg.

**Conclusion:** Findings from this study emphasize the need to enforce the use of ITNs and IPT in pregnancy in Nigeria especially ITN usage which is below average.
The possible role of nutritional status on the pro and anti-inflammatory cytokine balance of children with malaria from Imo State, Nigeria

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**Background:** Understanding the impact of malnutrition on pro and anti-inflammatory cytokine response in Plasmodium falciparum (p.f) infected children is very important for malaria control. This study specifically examined the balance between Interleukin 10, Tumour Necrosis Factor (TNF) and nutritional status of Plasmodium falciparum malaria infected children in South Eastern Nigeria to determine and evaluate the influence of malnutrition on immune response among p.f Infected children in Imo State Nigeria.

**Methods & Materials:** Ethical considerations were duly observed. Children aged 1-72 months with ongoing fever or history of fever within the last 24 hours and with no sign suggestive of severe malaria were involved in this study. Blood films stained with giemsa and rapid diagnostic test (RDT) kit were used to diagnose malaria parasitemia. Their Nutritional status was determined using the international Reference Population defined by the U.S National Center for Health Statistics (NCHS). IL-10 and TNF were assayed by ELISA. Statistical analysis was done using SPSS version 17.

**Results:** A total of 1344 febrile children were involved in this study. From this group 26.3% and 31.5% were positive for malaria parasites microscopically and through the RDT kit respectively. The cytokines were associated significantly with malaria infection. IL-10 / TNF ratio was significantly associated with parasite density and age (p<0.05). IL-10 / TNF ratio was lower in children 1-24 months when compared with older children. There was however no significant association between the nutritional status of these children and malaria infection. Non significant proportions were stunted (22.9%), Under weight (9.4%) and wasted (5.2%). Stunting, underweight and wasting cut across the study population. These findings show no significant relationship between presence of malaria and stunting, Underweight and wasting, rather stunting and wasting were associated with age (p< 0.05). There was no relationship observed between the IL-10 and TNF cytokines levels and the nutrional status of the study population.

**Conclusion:** Findings from this study suggest that there is no association between malnutrition and malaria as well as between the nutritional status of the study population and their cytokine level.
Background: Leishmaniasis is a neglected tropical disease caused by parasitic protozoa of the genus *Leishmania*. It has a spectrum of manifestations including cutaneous, mucosal and visceral disease. The clinical outcome of infection in humans is determined primarily by the infecting species and the immune response by the host. Sri Lanka is endemic for localised cutaneous leishmaniasis (LCL) caused by *Leishmania donovani*; a species which usually causes visceral disease. The aim of this study was to characterize the immune response in LCL by macrophages; the cells responsible for survival as well as eventual elimination of the parasite.

Methods & Materials: Peripheral blood mono nuclear cell (PBMC) derived macrophages from newly diagnosed LCL patients (n=8) and healthy non endemic controls (n=8) were stimulated with *L. donovani* antigen (50µg/ml) *in vitro*. The production of IL-10, TNFα and Nitric Oxide (NO) were measured by ELISA and Griess reaction at predetermined time intervals. The differences between experimental groups were analysed using the Student's t-test for parametric data and Mann-Whitney test for non-parametric data.

Results: Macrophages from patients produced more cytokines and NO at all time points. IL-10 production by patient macrophages was significantly higher (105.68 ± 26.05 vs 19.81 ± 28.24 pg/mL; p<0.01) at 72 hours but did not vary markedly at 24 and 48 hours. TNFα production by patient macrophages was significantly higher at both 24 hours (23.05 ± 13.97 vs 4.01 ± 2.26 pg/mL; p<0.01) and 48 hours (311.33 ± 206.29 vs 17.61 ± 21.09 pg/mL; p<0.01). Levels of production of NO remained similar at 24 and 48 hours but showed increased levels by patient macrophages at 72 hours (5.40 ± 1.15 vs 2.36 ± 1.21 pg/mL; p<0.01).

Conclusion: These data suggest that IL-10, TNFα and NO play a role in determining disease outcome in LCL due to *L. donovani*. In contrast to TNFα, the contribution of IL-10 and NO appear to be later in the infection. The findings should be interpreted in the context of changes in other inflammatory mediators, to better understand the underlying pathogenic mechanisms where a visceralizing *Leishmania* species is localized to the skin.
Novel cysteine desulphurase interacting protein Isd11 from Leishmania donovani: identification and the role in Fe-S cluster biogenesis

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**Background:** Iron-sulfur (Fe-S) clusters are essential and labile cofactors of proteins that facilitate central metabolic processes in all organisms. The Iron-Sulfur Cluster (ISC) system performs the general house-keeping function in Fe-S cluster biogenesis in bacteria and higher eukaryotes. *Leishmania* spp. has retained the ISC system, and for this reason we decided to study the ISC system and its components. However, no information regarding the role of Isd11 or its regulation is available for the *Leishmania* parasite an evolving pathogen model with rapidly developing drug resistance.

**Methods & Materials:** We expressed and purified recombinant *Leishmania donovani* (rLd) Isd11 protein by affinity column chromatography. Subcellular localization was performed using digitonin fractionation and immunofluorescence. We also checked the *in silico* protein-protein interaction and carried the physical interaction between Ld-Isd11 and Ld-IscS proteins through pull down assay and immunoprecipitation. Further, cysteine desulphurase (CDES) activity was assayed by monitoring sulphide production at 670 nm. Furthermore, the activities of rLd-IscS and rLd-LdIsd11 complex were assayed by adding free L-cysteine up to 10 mM and monitored by UV-Visible absorbance spectrometry. Lastly, we measured the emission spectra of IscS both in isolation and in complex of Isd11 by fluorescence spectroscopy.

**Results:** We found Ld-Isd11 protein to be localized within the mitochondrion of the *L. donovani*. Interaction of Ld-Isd11 with Ld-IscS was confirmed by pull down assay and immunoprecipitation which showed the involvement of Ld-Isd11 in Fe-S cluster biogenesis within the mitochondria. Following the displacement reaction upon the addition of L-cysteine, UV-Visible data showed that Ld-IscS/Isd11 complex follows the same spectral line as Ld-IscS alone. However, intrinsic fluorescence measurement showed the structural effect of rLd-Isd11 on rLd-IscS possibly by inducing change in the conformational state and thus Ld-Isd11 suggested to act as a stabilizer of Ld-IscS, sulfur donor/known to be upregulated in repair of ROS damaged Fe-S clusters.

**Conclusion:** We report the first characterization of a novel accessory protein, Ld-Isd11 which is involved in the mitochondrial Fe-S cluster biogenesis. However, Isd11 is absent from prokaryote genome which implies its supplementary role in eukaryotes. Thus, Ld-Isd11 forming a complex with Ld-IscS is an essential earlier step for Fe-S formation and may control the activity of Ld-IscS.
Association between BCG scars and risk of tuberculosis transmission among household contacts of active tuberculosis patients

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**Background:** Recognized risk factors do not explain the full variability of transmission for tuberculosis. Analyses of other host and environmental risk factors are required, including the bacille Calmette–Guérin (BCG) vaccine, whose effect for tuberculosis transmission is currently understood to be limited.

**Methods & Materials:** Between September 2009 and August 2012, we identified and enrolled active tuberculosis patients in Lima, Peru. Within one month of enrollment of these index patients, study nurses visited patients’ households to enroll their household contacts. We assessed the number of BCG scars on the household contact, as well as other risk factors for infection specific to the household contact, index tuberculosis patient, or the household. Household contacts underwent tuberculin skin testing (TST) to determine tuberculosis infection status at enrollment and at follow-up visits at six and twelve months, as appropriate. We used generalized estimating equations to model the association between number of BCG scars on the household contact and tuberculosis transmission as measured by (a) TST positivity at baseline and (b) TST conversion during follow-up.

**Results:** Among 10,314 household contacts of 2,700 index patients, 4,204 (40.8%) contacts were TST-positive at enrollment. After adjusting for other risk factors, we found that household contacts with three or more BCG scars had 19% increased risk of being TST positive, compared to household contacts without any BCG scars (adjusted RR, 1.19; 95% CI, 1.07–1.31; \( P_{\text{trend}} < 0.001 \)). During the 12-month follow-up period, household contacts with three or more BCG scars had 72% increased risk of becoming TST positive every six months, compared to household contacts without any BCG scars (adjusted RR, 1.72; 95% CI, 1.30–2.3; \( P_{\text{trend}} < 0.001 \)).

**Conclusion:** This is one of the largest cohort studies to date focusing on tuberculosis transmission. Our analyses suggest household contacts with more BCG scars are more likely to become TST-positive in the context of exposure to active tuberculosis patients. However, our analyses to date cannot definitely distinguish whether this reflects increased susceptibility to tuberculosis infection or another form of BCG boosting, which remains the subject of future research.
Predictors of adherence to isoniazid preventive therapy in HIV patients in Ethiopia: A prospective cohort study

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Background: Isoniazid preventive therapy (IPT) is efficacious in prevention of tuberculosis (TB) in Human Immunodeficiency Virus (HIV) infected persons. Yet, patients’ adherence to this strategy is suboptimal, its determinants are largely unknown and data are lacking from prospective cohorts. This study aimed to identify predictors of HIV patients’ adherence to IPT.

Methods & Materials: This prospective cohort study was conducted in the HIV/AIDS chronic care unit of Dilla University Hospital, Ethiopia, from May 2014 to February 2015. Adherence was defined as completion of the 6-month course of treatment with 90% of pills taken (measured by diary and pill count). Data was collected on potential predictors including patients’ demographic and clinical characteristics. Univariable and multivariable logistic regression models were fitted to identify independent predictors of adherence to IPT. The discriminative ability of logistic regression models was assessed by the area under the Receiver Operating Characteristic (ROC) curve and corrected for over-optimism using bootstrapping techniques.

Results: 162 HIV patients were included and 104 (64.2%) were adherent to IPT. In the final multivariable model, concomitant use of antiretroviral therapy (ART) and/or prophylactic cotrimoxazole therapy was associated with adherence to IPT [OR= 2.66; 95% CI (1.15, 6.17)]. Experiencing high level of HIV stigma and episodes of opportunistic infections tended to be associated with non-adherence to IPT [OR=0.51; 95% CI (0.25, 1.04)] and [OR=0.14; 95% CI (0.02, 1.15)]. The optimism-corrected area under the ROC curve of the final model was 67% [C index=0.67; 95% CI (0.57, 0.74)]. The calibration plot showed reasonable calibration.

### Table 2. Multivariable analysis of predictors of IPT adherence in HIV patients of Dilla University hospital

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Multivariable OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concomitant ART or co-trimoxazole</td>
<td>2.66 (1.15, 6.17)</td>
</tr>
<tr>
<td>Opportunistic infections</td>
<td>0.51 (0.25, 1.04)</td>
</tr>
<tr>
<td>HIV Stigma &amp; Discrimination</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.15 (0.02, 1.37)</td>
</tr>
<tr>
<td>High</td>
<td>0.14 (0.02, 1.15)</td>
</tr>
</tbody>
</table>

Conclusion: HIV patients receiving ART or cotrimoxazole therapy were more likely to adhere to IPT. We recommend studies with larger sample size to assess the effect of other potential predictors and incidence of adverse drug reaction after IPT-ART concomitant therapies. Population level and health care provider-associated determinants of IPT uptake should be investigated.
Seventeen years of drug-resistant tuberculosis in Argentinian children
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Background: The emergence of strains of Mycobacterium tuberculosis (Mt) that are resistant to antimycobacterial agents (DR-TB) is a worldwide problem of which the magnitude has not been well described specially in pediatric patients. TB susceptibility surveillance and risk factors for DR-TB are important issues to consider at the moment of pediatric TB treatment. **Objective:** To assess the prevalence of DR-TB in children, and identify clinical features, risk factors, and outcome of pediatric patients with DR-TB.

Methods & Materials: Retrospective review of all mycobacterial samples send to microbiological evaluation from 1998 to 2015 at Hospital Pediatría Garrahan. We analyzed 46 patients (p), with microbiological confirmation of DR-TB. Resistance tests to first line drugs: Isoniazid (INH), Rifampicin (RMP) Pyrazinamide (PZ), Ethambutol (EMB), Streptomycin (STO) were performed on Lowenstein- Jensen medium and BACTEC 460 TB system. We defined DR-TB at those strains of Mt resistant to one or more first-line drugs. We divided into single or resistant to 2 or more drugs. In the latter group, we recorded the data in two mutually exclusive categories: multidrug resistant TB strains (MDR-TB, those resistant to at least INH+RMP), and resistant to 2 or more drugs (other than INH+RMP).

Results: We included 1195 TB p, 478 bacteriologically positive confirmed culture. Forty six strains (9.6%) were DR-TB. Primary resistance was found in 39/46 (85%). The median age was 130 months (r: 2-204), 16 (35%) were immunocompromised, 75% of whom HIV positive. Pulmonary disease was the most common presentation: 29 p (60%). Eighteen p (40%) had extra-pulmonary localization. Of 46 DR-TB cultures 28 (60%) were monoresistant and 18 (40%) resistant to 2 or more drugs. Ten were MDR-TB 8 of who had HIV infection. Monoresistance for INH, RMP, PZ, EMB and STO were 1.6% 0.2%, 0%, 0.2% and 4% respectively. Resistance to INH was 3%. Fourteen p (30%) had TB contact, 9 (19%) had previous hospital admission, and 7 (15%) previous TB treatment. Three p (6%) died because of TB, all of them with central nervous system infection.

Conclusion: DR-TB is infrequent in pediatric patients. MR-TB is associated with HIV. INH resistance was present in 3% of all isolated strains.
Early cardiac safety of the 9-11 month Short course regimen for MDR-TB treatment

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Background: Only 48% of multidrug resistant tuberculosis (MDR-TB) patients worldwide receive successful outcomes. Shorter, more effective regimens are required. The cardiac safety of the 9-11 month “Bangladesh regimen” has been largely unstudied. The regimen was piloted in Uzbekistan and as per WHO recommendations, electrocardiographic (ECG) monitoring was performed in the early phase of treatment. We aim, in this interim analysis, to describe the early cardiac safety of this regimen.

Methods & Materials: Consecutively diagnosed MDR patients were recruited from Karakalpakstan, Uzbekistan. Moxifloxacin 400mg and clofazimine 100mg daily were included in the regimen. Patients had a single, 12 lead ECG performed at baseline, 2 and 4 weeks of therapy. RR and QT interval length was measured using lead II or V2 on the ECG printouts. QT was corrected using Fridericia’s formula (QTcF). Data was prospectively collected using a standardised database (Koch 6, MSF, Paris) and analysed using openEpi (Ver 3.03, accessed 26/7/2015).

Results: Between September 2013 and March 2015, 146 patients were enrolled. No patients were excluded from the study due to baseline cardiac findings or QT prolongation. Of these, 121 had ECGs performed per protocol and available for analysis. 27 (22.3%) were over 50 years of age and six had a reported history of cardiac disease. 12 patients (9.9%) had increases >60 ms from baseline with 1 patient showing an increase at both time points. Only one of these patients had QTcF increased over 450 ms. No patients had QTcF >500 ms during the screening period. No patients reported syncope or seizures and there were no deaths during the screening period. Univariate analysis of those with QT prolongation versus no QT prolongation did not identify other significant risk factors.

Table 1 - ECG data

Table 2 - Univariate analysis of cohort with QT prolongation vs. no QT prolongation

Conclusion: Our results suggest that a 9-11 month regimen has acceptable early cardiac safety. As expected, QTcF is prolonged, the significance of which in MDR-TB patients requires further investigation.
Detection of mycobacterium leprae in tissue sections using auramine O fluorescent stain versus modified fite-faraco: A comparative study
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Background: Modified Fite-Faraco method is commonly used for detection of lepra bacilli in the tissue samples. Recently, fluorescence microscopy have provided enhanced sensitivity and specificity over those of the conventional techniques especially when lepra bacilli numbers in tissue specimens were low. The present study was initiated to compare conventional Modified fite-faraco based detection of lepra bacilli detection with Auramine stain-based fluorescence microscopy.

Methods & Materials: One hundred eighteen skin biopsies was obtained from patients clinically diagnosed as leprosy. Disease was classified into Indeterminate (/)(n=28), Tuberculoid (TT=2), Borderline tuberculoid (BTH,n=67), Borderline borderline (BB,n=2), Borderline Lepromatous (BL)(n=13) and Lepromatous (LL,n=6). Each biopsy was stained by Auramine and Modified Fite-Faraco. The sections was screened for the detection of lepra bacilli. Sections stained by Auramine was seen under Fluorescent microscope which showed bright yellow rods against dark background.

Results: Out of 118 biopsies, 75 were positive by Auramine while only 41 by Modified Fite-faraco. Out of 28 Indeterminate cases 12(42.9%) showed bacilli by Auramine and 2(7.1%) by Modified Fite-Faraco. 42(62.7%) out of 67, and 20(29.9%) out of 67 BTH patients were positive by Auramine and Modified Fite-Faraco respectively. Biopsies from BB were positive by both the methods. In BL/LL Modified Fite-Faraco detected bacilli in 11 out of 13(84.6%) and all 6(100%) respectively while Auramine detected bacilli in all 19(100%)BL/LL biopsies.

Conclusion: Auramine and Modified Fite-Faraco are equally sensitive for detecting lepra bacilli in lepromatous pole. The Auramine method was found to be superior to Modified Fite-Faraco especially in early spectrum of leprosy.
Curvilinear scars indicator of Lucio’s phenomenon in leprosy
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Background: Lepromatous leprosy may present two types of vasculonecrotic reactions. Lucio’s phenomenon and that associated with ENL. We present a case of lepromatous leprosy that developed Lucio phenomenon and ENL.

Methods & Materials: A 28yr old male complained of asymptomatic red raised lesions over body since 1 ½ months which subsided with atrophic scars. He had past history of recurrent evanescent painful nodules. On examination multiple curvilinear jagged atrophic scars with surrounding halo of hypopigmentation were seen over arms, forearms, and legs. Multiple thickened nerves were present. Sensations were normal except for decrease in temperature and hot-cold on medial aspect of the left palm.

Punch biopsy from the margin of a scar showed leukocytoclastic vasculitis, thrombosed blood vessels, infiltration of histiocytes, Langerhans giant cells in dermis, and AFB in endothelium on Modified Fite Faraco staining. Another punch biopsy from a newly developed nodule showed obliterative granulomatous medium vessel vasculitis with presence of AFB in endothelium on Modified Fite Faraco staining. Slit skin smear showed 4+ BI on average. After a month of starting MDT and systemic steroids, patient developed multiple ENL, which subsided with appropriate treatment.

Results: Based on characteristic curvilinear scars, small and medium sized vasculitis and presence of AFB globi in endothelium, a primary diagnosis of Lucio’s phenomenon was made. After a month of starting MDT and systemic steroids, patient developed multiple ENL, which subsided with appropriate treatment. So a final diagnosis was coexisting Lucio phenomenon and ENL in lepromatous leprosy.

Conclusion: Lucio’s Phenomenon is very rarely reported from India. We report this case to highlight the difficulties in differentiating LP from necrotic ENL.
New antibacterial agents targeting mycobacterial the ATP synthase
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**Background:** A set of 700 compounds found active after the whole cell screening of 20,000 compounds library against *Mycobacterium tuberculosis* H37Rv was screened in the ATP synthase assay. Three compounds showed promising IC50 and were further evaluated in the time kill assay and combination studies with first line anti-TB drugs against *M. tuberculosis* H37Rv. The compounds were tested for cytotoxicity against HepG2 cell lines.

**Methods & Materials:** ATP synthase assay was performed using the inverted membrane vesicles of *Mycobacterium smegmatis* for the screening of the 700 compounds by estimating the ATP production. In-vitro activities of the selected compounds were done by microdilution method, cell toxicity profile using MTT assay in HepG2 cell line, in vitro time kill assay and combinational studies with first line drugs.

**Results:** Three compounds C1-C3 showing promising inhibitory activity (IC50 values 0.13-4.0 µM) in the in-vitro ATP synthase inhibition assay also exhibited potent anti-mycobacterial activity (MIC-0.06-2.0µg/ml). These compounds were nontoxic in HepG2 cell line, Compound C1 was bactericidal at 8X MIC (1 µg/ml) and exhibited synergistic activity with rifampicin.

**Conclusion:** ATP synthase screening of whole cell active compounds from IIIM repository led to the identification of three compounds showing good inhibitory activity against ATP synthase. All the compounds displayed promising anti-mycobacterial potential with no detected toxicity against mammalian cell line. Compound (C1) showed synergistic activity with rifampicin, which forms the backbone of anti-TB therapy. These compounds represents novel chemotypes against ATP synthase and can be taken up for medicinal chemistry efforts.
Clinical profile and evaluation of diagnostic tests in culture positive childhood tuberculosis

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Background: Tuberculosis (TB) ranks among the ten major causes of mortality among children. The diagnosis of childhood TB remains quite challenging. Mycobacterial culture is the gold standard in diagnosis; however, positive cultures are seen only in 50%. The aim was to study the clinical profile and sensitivity of other diagnostic tests in culture-proven tuberculosis in children.


Results: There were 61 cases of culture-confirmed TB (32 boys and 29 girls). The mean age at diagnosis was 7.8 ± 5 years. History of contact with TB was present in 14 children. The major presenting symptoms included fever (78%), cough (49%), loss of appetite (36%) and loss of weight (24%). Pulmonary Tb was seen in 22 children (36%) and extra-pulmonary TB in 39(64%). Disseminated TB was seen in 46%. Two children tested positive for HIV. Results of Drug susceptibility testing was available for only 41/61 children. Four children had MDR TB; none had XDR TB. All children were started on daily anti-tuberculous therapy. Of the 61 children, 26 were cured, 26 children were referred elsewhere for treatment, and 9 children died or were discharged in a moribund state (14.8%). There was no significant difference in treatment outcome between the pulmonary and extra pulmonary group (p=0.393). The sensitivity of ESR was 96% and Mantoux was 69.2% in the overall group. In culture-confirmed pulmonary TB, sensitivity of chest X-ray was 95.5% and AFB smear was 45.5%.In culture-confirmed extra-pulmonary TB, sensitivity of AFB smear was 38.5% and that of biopsy was 25%.

Conclusion: The majority of culture-confirmed TB at this tertiary care center was extra-pulmonary TB, with a high incidence of disseminated disease. Drug-resistant TB was seen in only a few children. The sensitivity of AFB smear, Mantoux and biopsy in making the diagnosis was quite low. ESR was highly sensitive but this test is highly nonspecific and elevated in many diseases.
Pharmacists contributing to the WHO Stop TB theme: “Find, Treat, Cure Everyone"
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**Background:** Community Pharmacists, being first point of contact for people with symptoms are in the suitable position to detect the chest symptomatic cases. Since couple of years, pharmacists are being trained by Indian Pharmaceutical Association (IPA) jointly with Revised National Tuberculosis Control Programme (RNTCP) and with support of Lilly MDR TB Partnership. IPA has been working in state of Maharashtra in various districts as well as spreading this work to other states namely Madhya Pradesh, Goa, Tamilnadu and Gujarat.

**Methods & Materials:** Training manual jointly developed by IPA and Central TB Division, Ministry of Health and Family Welfare was used for training community pharmacists. Training sessions provided information on TB, DOTS protocols, role of pharmacists (how to identify a case, whom to refer, where to refer the case, how to use “Laboratory Form for Sputum Examination” (Referral Form), how to guide the case about medication) and the significance of communication skills. Copy of list of the Designated Microscopy Centers (DMCs) and list of contact details of RNTCP staff was given to each participant pharmacist. Interactive sessions for RNTCP field staff and pharmacists were organized to develop effective partnership.

**Results:** As per the data from April 2014 to April 2015, IPA has trained 438 pharmacists. Post training, pharmacists were followed up by IPA and were encouraged to refer chest symptomatic cases. Out of 180 referred cases, 24 were found to be TB cases. The training was proven as quite effective in finding out the cases from the community. Most of the trained pharmacists started referring the cases to nearby DMCs yet quite few cases didn't reach the DMCs due to various reasons. The pharmacists' work was continuously acknowledged by media boosting their spirits up.

**Conclusion:** Pharmacists, if trained and followed up effectively, can reach the unreached and find out the chest symptomatic cases coming to the pharmacy from the community. Pharmacists have huge potential for contributing to the WHO stop TB theme; "Find, Treat, Cure Everyone". RNTCP should engage all care providers including pharmacists for TB prevention and control.
Prevalence, risk factors and clinical outcomes of Xpert MTB/RIF identified rifampicin-resistant tuberculosis in Bukavu, Democratic Republic of the Congo

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Background: Little is known on prevalence, risk factors and clinical outcomes of rifampicin-resistant tuberculosis (RR-TB) in Bukavu.

Methods & Materials: We conducted a secondary analysis on a database containing clinical and RR-TB data collected cross-sectionally using the Xpert MTB/RIF assay at Provincial General Referral Hospital (PGRH) of Bukavu. The primary outcome was RR-TB prevalence. A multivariate logistic regression was performed to identify independent risk factors of RR-TB.

Results: Of 225 participants analyzed, 65.8% were male. The mean (SD) age was 34.9 (16.8) years. 27 of 85 patients (12.0%) with HIV were on antiretroviral therapy. 167 (74.2%) patients had pulmonary tuberculosis (TB); 131 (58.2%) of these were TB sputum smear-positive. 85 (37.8%) were HIV positive. Of note, 45 (20%) participants were TB retreatment cases. The prevalence of RR-TB was 8.9% (95% CI 5.5 – 13.4). Overall, 28 (12.4%) deaths were recorded of which 6 deaths (30%) occurred in RR-TB group (P= 0.013), 3 (75%) deaths among 4 HIV positive and RR-TB co-infected patients (P= 0.03). Risk factors independently associated with RR-TB were: retreatment cases vs. new TB cases (adjusted odds ratio [aOR] = 5.5, 95% CI 0.9 – 32, P=0.06); and having a previous TB treatment failure ([aOR] = 17.6, 95% CI 2.2 – 142, P=0.007).

Conclusion: We are documenting a relatively high prevalence of RR-TB in patients at PGRH of Bukavu and the major risk factor being previous TB therapy failure. Both primary and acquired TB drug resistance are common in this setting, therefore, there is a need for strengthening the quality of the TB control program in this setting. This can be achieved through increased availability and expansion of diagnostic services, including Xpert MTB/RIF and other drug susceptibility testing facilities to be used when TB is suspected and/or diagnosed. This will assist in optimizing the suitability of RR-TB treatment regimen. Finally, there is an urgent need for the development of effective drugs for the management of RR-TB.
Hospital based prospective observational case study to evaluate the prevalence of diabetes mellitus among tuberculosis patients in a tertiary care hospital, in India

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Background: Diabetes mellitus (DM) is recognised as an important risk factor to tuberculosis (TB). India has high TB burden, along with rising DM prevalence. The recent change in life style of people of India and westernization of food habits has contributed much to make India the diabetes capital of world. Rapid urbanization has resulted in crowding of cities and has led to rapid spread of infections. So in a majorly immunocompromised India because of DM, TB is a common infectious disease. Thus DM and TB affect each other. In both the diseases the number of cases reported at tertiary care centres is just the tip of iceberg. We conducted an observational study at, Apollo Hospital, Hyderabad, India to look for prevalence of TB among DM patients.

Methods & Materials: Patients older than 18 years with TB and not otherwise immunocomprised were considered for the study. All sputum positive, sputum negative and extra-pulmonary cases currently on anti-tuberculosis treatment and newly diagnosed were included in the study that were admitted as inpatient or reported as outpatient at Apollo Hospital in department of Medicine. TB patients were screened for DM through a thorough history, detailed examination and lab investigations.

Results: Seventyfour patients met the criteria and were included in the study. In our study 24 of total 74 i.e.(32.43%) study patients were found to be diabetic; mean age was 46 ± 17.8, (males 48.5 ± 17.4 and females 44.3 ± 19.5); 61% were male. Among the diabetic population 80 % were male (p value 0.0407). 11 of 24 DM patients were newly diagnosed which is 46% and 13 patients (54%) were known diabetics. 35 patient (47%) were suffering from pulmonary tuberculosis and 39 (53%) from extra-pulmonary tuberculosis.

Conclusion: High prevalence of DM among TB patients was found in or study at a tertiary care hospital in India. The prevalence of DM was more in patients with pulmonary TB, among males and patients with history of smoking. We recommend screening for DM among people with TB and vice versa, in a country like India with a high double burden of disease.
Role of PCR for diagnosing male genital tuberculosis

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**Background:** Diagnosis of male genital tuberculosis is difficult because clinical features and imaging findings are non-specific and conventional laboratory methods are time-consuming and almost non-contributory. It is important to have the diagnostic method that has high sensitivity and specificity. Hence, this study was done to see the utility of tissue and semen PCR in diagnosis of male genital tuberculosis and its comparison with histopathological examination (HPE).

**Methods & Materials:** A prospective observational study was done from Jan 2006-Dec 2014 in Department of Urology and Microbiology of Kasturba Medical College, Manipal. 74 tissue samples (Epididymis 49, Prostate 20 and Periurethral 5) of suspected cases of male genital TB were processed for both HPE and PCR. 15 semen samples from patients with hematospermia were processed for only PCR.

**Results:** 32 tissue samples (22 epididymis and 10 prostate tissues) were positive for both HPE and PCR whereas 36 samples were negative for both tests. False positive and false negativity was observed in 4(5.4%) and 2(2.7%) samples respectively. Considering HPE as gold standard, PCR showed the sensitivity and specificity as 88.9% and 94.7% respectively with kappa agreement as 0.8. Time taken for PCR results was about 3.1 days as compare to 6.2 days for HPE. Semen PCR showed positivity for 4 samples (26.7%).

**Conclusion:** Tissue PCR is a sensitive and specific method for obtaining early and timely diagnosis of male genital tuberculosis. Semen PCR adds qualitative benefit for diagnosing tuberculosis in male genital tract.
Intranasal delivery of antituberculosis agents in a murine tuberculosis model
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Background: The use of aerosol delivery of antimycobacterial agents for the therapy of tuberculosis in mice has not been well studied. Aerosol therapy in mice is problematic due to the high cost of the required apparatus. The purpose of this study was to explore the efficacy of intranasal (IN) administration (as a surrogate for aerosol delivery) of isoniazid (INH) and rifalazil (RZL) in a murine tuberculosis model compared to oral delivery.

Methods & Materials: Six week old female Balb/c mice (purchased from Charles River Laboratories, Wilmington, MA) were infected intranasally with about $10^3$ CFU of *Mycobacterium tuberculosis* (MTB) ATCC 27294 (H37Rv) for experiment 1 or about $10^6$ CFU of MTB ATCC 35801 (Erdman) for experiment 2. One week post infection mice in experiment 1 were treated with INH 5mg/kg orally by gavage or 5mg/kg IN for 3 days and mice in experiment 2 were treated with RZL 5 mg/kg orally by gavage 5 days/week or 5 mg/kg IN Mon, Wed, and Fri for 2 weeks. At the initiation of therapy in each experiment a group of mice (early controls) were euthanized by CO₂ inhalation and their lungs were collected. At the completion of therapy an untreated group of mice, late controls (LC), and treated mice were euthanized. Mycobacterial loads in right lungs were measured by serial dilution and plating on Middlebrook 7H10 agar plates.

Results: The mycobacterial loads (log CFU) for the EC, LC, INH oral and INH IN were 3.34, 4.49, 2.94, and 2.82 respectively (exp. 1). The mycobacterial loads for the EC, LC, RZL oral, and RZL IN were 6.26, 8.93, 4.30, and 4.58 respectively (exp. 2). The LC group was euthanized 3 days early due to their advanced illness. IN delivery of INH and RIF was significantly better than the untreated late controls.

Conclusion: The activities of INH and RZL by IN delivery in these experiments suggest that other agents that cannot be given orally could be evaluated for their potential therapeutic activities by IN administration.
Real-time PCR of whole blood specimens transported in PrimeStore MTM® to detect and monitor MTB bacteremia

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**Background:** *Mycobacterium tuberculosis* (MTB) is an important cause of bacteremia and sepsis in HIV patients residing in sub-Saharan Africa. Many patients with MTB sepsis go undiagnosed and die within 18 days of presentation, making culture inadequate for detecting MTB in the blood. **Objective:** To determine the feasibility of ambient temperature transport of blood in PrimeStore MTM® to a distant lab for real-time PCR detection of MTB bacteremia and to monitor clearance of MTB from the blood after therapy.

**Methods & Materials:** BALB/c female mice were injected intravenously with 0.2 mls of ethanol killed MTB (approximately $10^5$ CFU/mL). Two anti-MTB opsonophagocytic bactericidal MABs were used to simulate treatment of MTB sepsis. Mouse monoclonal antibodies (MAB LHN-AB9 or LHN-GG9) or control were given IP using 0.3 mls of sterile PBS 24 hours before MTB challenge. To monitor MTB in the blood, mice were bled at 3 time points: immediately after injection with MTB and again at 4 and 24 hours after MTB challenge. Collected blood was placed into citrate tubes and 0.1ml was transferred into PrimeStore MTM®. Samples were transported at Ambient temperature from Gaithersburg, MD to San Antonio, TX. DNA was extracted from blood in PrimeStore MTM® and replicate real-time polymerase chain reactions (PCR) were performed using PrimeMix® MTB Complex on an ABI 7500 Instrument.

**Results:** Blood PCRs on specimens obtained 15 minutes after MTB challenge were positive with an average C_T value of 29.8 (range 29.2-30.6). Mice treated with PBS control had MTB detected in the blood by PCR at all time points (at 15 min, 4 and 24 hours post challenge). Mice given anti-TB opsonic MABs cleared the MTB from the blood either by 4 or 24 hours ($C_T=40$).

**Conclusion:** Blood specimens were efficiently transported to a central lab to detect MTB bacteremia by PCR. In addition, PCR may be useful to monitor patient treatment, similar to viral load testing for HIV. Using PrimeStore MTM® to ship specimens safely and rapidly at ambient temperature to a regional facility for PCR analysis may provide rural hospitals in sub-Saharan Africa the opportunity to diagnose MTB sepsis and monitor treatment.
GeneXpert detection of mycobacterium tuberculosis from sputum collected and transported in a molecular transport medium

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Background: The Cepheid GeneXpert (Xpert) System is a WHO-endorsed, widely utilized molecular diagnostic platform for Mycobacterium tuberculosis (MTB) and rifampin (RIF) detection. In spite of global success, several challenges remain, especially the need for safe, cost-effective transport/shipping, and improved MTB detection sensitivity. PrimeStore Molecular Transport Medium® (PS-MTM) was developed for specimen inactivation and DNA/RNA stabilization at ambient temperature for downstream molecular applications. Aims: Xpert MTB-RIF assay was compared to real-time PCR to evaluate detection of MTB and rifampin (RIF) in PS-MTM or PBS control medium over a dynamic concentration range. Furthermore, Xpert MTB/RIF detection was performed using clinical smear-positive sputum collected from swabs in PS-MTM and PBS control, or processed as raw sputum.

Methods & Materials: Ten-fold reductions of whole MTB (10⁴ to 1 CFU/mL) in PS-MTM and PBS control were analyzed using GeneXpert and real-time PCR on an ABI 7500. For Xpert evaluation of clinical material, 50-150 µL of smear-positive (3+to1+) sputum specimens (N=17) were transferred by flocked swab into PS-MTM and compared to equivalent amounts collected in PBS and routine Xpert testing according to manufacturer’s recommendation.

Results: Using Xpert, MTB from PS-MTM was detected at 10 CFU/mL compared to 10² CFU/mL MTB detected from PBS controls. Overall Xpert PCR efficiency from PS-MTM (63.2%) was improved compared to PBS controls (34.9%). In Xpert, C₇ values from higher MTB concentrations in PS-MTM were increased compared to control; however PS-MTM showed superior detection from low level MTB concentrations. Xpert assay detected MTB from sputum collected by flocked swabs placed in PS-MTM in 17 of 17 specimens and corresponded to routine Xpert detection using 1.0 mL of sputum. NGS of multi-drug resistance genes was performed from the volume remainder of five PS-MTM specimens. Resistance conferring mutations for rifampicin were noted from two specimens in the rpoB gene, which corresponded to Xpert rifampin-resistance detection.

Conclusion: PS-MTM enhances MTB detection when specimens contain low level MTB. Sputum collection in PS-MTM provides safe and inexpensive shipment/transport at ambient temperature to centralized testing sites. Small sputum volume collected using a flocked swab allows the remaining sample to be safely archived, re-tested, or evaluated for drug resistance by NGS.
Identification of Mycobacterium tuberculosis complex in clinical specimens of HIV-infected patients at Instituto de Infectologia Emilio Ribas, São Paulo-Brazil
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Background: Tuberculosis (TB) remains the most common infection among HIV patients. Currently, the TB diagnosis is still based on the clinical presentation, radiographic findings and microbiological results. Considering the complexities of treating HIV/TB co-infection, TB diagnosis requires the availability of diagnostic tools that allow the rapid detection of Mycobacterium tuberculosis complex (MTBC) and drug resistance in clinical samples.

Methods & Materials: In this retrospective study conducted at the Instituto de Infectologia Emílio Ribas, São Paulo/BR, we analyzed a total of 5350 clinical specimens (respiratory and extra-pulmonary) collected from patients with signs and symptoms suggestive of TB from January/14 to December/14. All samples were processed by conventional diagnostic techniques, including smear examination for acid-fast bacillus (AFB) and cultured in MGITF-960 automated system. Blood and bone marrow were cultured in BACTECFX. Identification of MTBC and non-tuberculous mycobacteria (NTM) was performed by rapid immunochromatographic assay. The average time needed for detection of mycobacteria was 15 days. Susceptibility testing for MTBC and PCR for NTM was performed by Adolfo Lutz Institute, S. Paulo.

Results: Of the 5350 samples, 554 (10.35%) were positive by culture for mycobacterial agents. Among the culture positive specimens, 428 (77.25%) were from HIV-infected patients, and 342 (61.73%) were collected from male. From the 554 culture-positive specimens, of which 398 (71.84%) respiratory and 156 (28.15%) extra-pulmonary, 391 (70.57%) had a positive rapid test for MTBC and 95 (17.14%) had a positive rapid test for NTM. From NTM, Mycobacterium avium complex (MAC) was the most prevalent (52.48%), followed by M. kansasii (15.78%) and M. fortuitum (5.26%). Resistance was identified in 25/391 MTBC isolates (6.3%), and the most frequently resistant drugs were rifampicin (44%) and isoniazid (32%), respectively.

Conclusion: TB is an important public health problem and the diagnosis in HIV-infected patients is challenging. The use of mycobacterial culture remains an important diagnostic tool. The immediate future involves rapid molecular techniques, in particular GeneXpert which is also able to detect rifampicin resistance. In order to improve diagnosis and detect as early as possible resistance to rifampin, it was introduced earlier this year the GeneXpert MTB/RIF in our hospital which proposes to be a strong diagnostic tool for pulmonary TB.
Cholecalciferol adjunctive therapy in active tuberculosis

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Background: Vitamin D enhances immune responses to tubercle bacillus. The aim of our study was to demonstrate whether or not use of cholecalciferol as supplement to patients with TB may be improved clinical outcome.

Methods & Materials: Sixty patients with pulmonary tuberculosis were randomised to take either 450000 International Units of cholecalciferol or placebo. Evaluation were carried out at one, two and three months later. The first outcome was reduction in TB score and the secondary outcome was smear conversion and improvement of quality of life. Analyses were conducted using SPSS software (ver. 18) according to a pre-specified plan.

Results: Mean calcidiol levels for the whole study population were within the insufficient range (22.81 ± 10.76 ng/ml). There have been no associations between baseline calcidiol levels and sputum smear burden (P-value = 0.54). There was an association of TB severity score with lower levels of Vitamin D (P-value = 0.043). The general social functioning (SF)-12 health survey scoring at enrolment in two arms did not differ significantly (P-value = 0.786). However two months later findings indicate that 25-hydroxyvitamin D treatment had a positive effect on progressing health-related quality of life (P-value = 0.019) in each subscale of physical health score (P-value = 0.028) and mental health score (P-value = 0.025).

Conclusion: Our findings indicated that high dose cholecalciferol supplementation can lead to improve clinical outcome in tuberculous patients especially in patients with calcidiol deficiency. Tuberculosis alleviate quality of life and necessary at TB clinics to apply strategies to improve the health-related quality of life of TB patients. Therefore, we recommend vitamin D supplement therapy for this purpose.
Mycobacterium tuberculosis acetyltransferase reduces the oxidative stress response through expression of peroxisomal membrane transporter protein

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**Background:** *Mycobacterium tuberculosis* (M.tb) survives inside the macrophages by manipulating the host immune responses. Mtb cell-wall associated glycoproteins play an important role in initiation of host-pathogen interactions.

**Methods & Materials:** *M.smegmatis* mc2155 was used in this study. Cloning and expression was performed in pSMT3 shuttle vector. Invasion assay in HeLa cells. Survival assay, autophagy, oxidative stress response, immunostaining by fluorescence microscope and Western blot analysis was done in mouse macrophages. Microbial adhesion to hydrocarbons (MATH) test was performed to assess bacterial hydrophobicity. Bacterial susceptibility against cell wall acting antibiotics was done with CFU (Colony Forming Unit) assay.

**Results:** To identify novel Mtb glycoproteins, we employed a multi-lectin system to capture glycoproteins from purified Mtb cell wall and identified them by mass spectrometry analysis. A novel protein as putative acetyltransferase (ACTase) was identified. Recombinant *M.smegmatis* expressing the ACTase (MsmACTase) showed increased invasion in human epithelial cells and survival in mouse macrophages. Increased intracellular bacillary burden was a result of inhibition of autophagy and ROS production due to reduced expression of superoxide dismutase (SOD) and catalase enzymes in Msm ACTase infected macrophages when compared with wild-type and vector control (pSMT3) strains. Subsequent studies showed that decreased ROS production was due to over expression of ROS scavenging peroxisomal membrane protein 70 (PMP70). MsmACTase showed increased expression of acylCoA oxidase (ACOX-1), a classical marker enzyme for peroxisomal β-fatty acid oxidation. MsmACTase also exhibited increased production of nitric oxide and expression of inducible nitric oxide synthase (iNOS) in infected macrophages. Moreover, MsmACTase showed increased resistance to cell wall acting anti-TB drugs and to lysozyme due to the increased cell surface hydrophobicity.

**Conclusion:** We have shown that acetyltransferase gene (ACTase) of *Mtb* expressed in *M.smegmatis* aid intracellular mycobacterial survival through inhibition of autophagy and oxidative stress responses in macrophages. The present study reports for the first time that Msm ACTase scavenges H\textsubscript{2}O\textsubscript{2} due to over expression of ROS scavenging peroxisomal membrane protein 70 (PMP70) with which insights a new mechanism how the pathogen surpass the host defense in *Mtb* infection. The above findings may lead to identification of a potential drug target for the antimycobacterial therapy.
Analysis on direct medical costs and compensation for whole course of treatment of pulmonary tuberculosis patients in Shanghai

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Background: To describe the overall direct medical costs and the compensation from health insurance systems and the TB control projects, and analyze the economic burden for the whole course of treatment of pulmonary tuberculosis (PTB) patients in Shanghai.

Methods & Materials: The copy of invoices during the course of TB diagnosis and treatment of 766 newly registered PTB patients from 2013 were collected in four districts in Shanghai, meanwhile these patients were investigated by questionnaire. Descriptive analysis and ranksum test were employed.

Results: The medians (inter-quartile range) of 766 PTB patients' direct medical costs for whole course of treatment were 5757.4 (3749.87, 12632.98) yuan/person. All patients had an out-patient cost with a median of 4605.3 (3490.8, 6335.2) yuan/person, consisted of the cost of western medicine (47.6%), Chinese patent drugs (19.9%), laboratory test fee (18.1%), inspection fee (12.1%) and treatment/registered fee (2.3%). Among the western medicine fee, hepatoprotectants accounted for 57.1%, followed by the first-line anti-TB drugs (20.2%) and second-line anti-TB drug (11.1%); a higher proportion of hepatoprotectants in Chinese patent drugs, reached 60.8%; in laboratory test fee, costs of liver function tests (36.2%) and tuberculosis determination (31.5%) were relatively higher; inspection fee was mostly CT cost, accounting for 82.9%. The amount of cost was related to the District, age, the location of household register, health insurance types, the level of medical institutions, category of PTB. The median of the proportion of health insurance reimbursement from total cost was 8.8%, the proportion of TB control project reimbursement from total cost was 33.8%, out-of-pocket payment was account for 44.8% of total cost and 5.8% of disposable household income in 2012. But there was still about 6.5% of PTB reached catastrophic health expenditure (>40% of disposable income).

Conclusion: The direct medical costs of whole course of treatment for PTB patients in Shanghai were relatively high, especially the costs of hepatoprotectants, liver function laboratory test and CT examination. Current TB control project reduced the economic burden of PTB patients in a certain extent, but the item and proportion of reimbursement, and the procedure of the cost reduction still need to be optimized.
Confirmation of silent mutations in the rpoB gene locus of M. tuberculosis isolates using pyrosequencing and phenotypic DST

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**Background:** More than 95% cases of rifampicin resistance in M. tuberculosis strains can be attributed to mutations in 81-bp hotspot region of rpoB gene locus called Rifampicin Resistance Determining Region. These mutations can be detected by rapid molecular diagnostic techniques such as Line Probe Assay (LPA), but detection of silent mutations on LPA may not necessarily confer rifampicin resistance and result in false rifampicin resistance reporting. These silent mutations can be confirmed by phenotypic Drug Sensitivity Testing (DST) and/or by DNA sequencing.
Following study was undertaken to confirm silent mutations using phenotypic DST (Gold standard) and pyrosequencing, a rapid real time method for sequencing small DNA segments by synthesis.

**Methods & Materials:** Total 300 DNA extracts comprising of 101 silent mutation strains; 101 pan-sensitive, 96 MDR-TB and 02 rifampicin mono-resistant strains were processed for pyrosequencing following detection by LPA.
Pyrosequencing was performed with sequence analysis mode of PyroMark Q96 ID system (Qiagen, Valencia, CA).
All 300 sputum concentrates were also processed for phenotypic DST, using solid and liquid culture methods.

**Results:** Pyrosequencing detected mutations in all 101 silent mutation strains, but 02 strains did not produce amino acid change (true silent mutations) and were also rifampicin sensitive phenotypically. Maximum mutations were seen in 526 codon (35 strains) followed by 511 codon region (30 strains). Two novel mutations were reported: substitution in 529 codon CGA → CAA and 517-518 codon deletion.
However, only 51/99 true rifampicin resistant strains on Pyrosequencing were rifampicin resistant by MGIT 960 and 57/99 by solid DST. This proves that low level rifampicin resistance linked to specific rpoB mutations could be missed by phenotypic DST and this could be attributed to the critical concentration of rifampicin used.
101 pan-sensitive, 96 MDR-TB and 02 rifampicin mono-resistant strains used as controls were confirmed by pyrosequencing and showed 100% concordance with phenotypic DST.

**Conclusion:** Pyrosequencing is a confirmatory tool for detection of rifampicin resistance in silent mutation cases in M. tuberculosis and reiterates the fact that liquid DST may miss some rifampicin resistance compared to solid DST conferring mutations further suggesting that the gold standard for rifampicin resistance be reconsidered. Treatment outcome with these mutations is not well known.
Phytochemical and antimycobacterial analysis of aqueous and ethanolic extracts of Annona muricata Linn (Soursop)
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Background: Against the backdrop evidenced in the threat Tuberculosis poses to developing economies, especially its prevalence among people in their productive (15-45) years; this preliminary study examined the phytochemical constituents and antimycobacterial effect of four (4) aqueous and ethanolic extracts from the fruit skin (epicarp) and leaf of Annona muricata Linn.

Methods & Materials: Extracts were prepared with distilled water and 95 % ethanol according to methods previously described. Phytochemical analysis of the extracts were carried out following standard protocols while the antimycobacterial activity was assayed by employing the Drug susceptibility testing (DST) procedure in a Biosafety Level 3 facility. Lowenstein Jensen (LJ) media were prepared with extracts at three concentrations (1, 40 and 250 µg/ml) following the project design and subsequently inoculated with 10⁻³ and 10⁻⁵ suspensions of both control (H37Rv) strain and a clinical isolate (MTB-584) of Mycobacterium tuberculosis. LJ media prepared with Rifampicin at 40 µg/ml was used as the standard drug for positive control while plain media with respective inoculum served as negative control. Four Ziehl-Neelsen’s stain slides were also prepared to confirm the presence of organisms in the two suspensions employed for the two strains tested. Plain media inoculated with distilled water were employed as normal control to check for possible contaminant. The inoculated media and control slants were placed in an incubator at 37°C and observed every seven days for a period of 4 – 6 weeks.

Subcultured strains of MTB on LJ slants

Preparing LJ for DST

Results: The phytochemical analysis collectively revealed the presence of tannins, saponins, flavonoids, anto- and betacyanins, terpenoids, phenols and steroids. The M. tuberculosis strains exhibited resistance to all the four extracts at tested concentrations as there was substantial growth with typical creamy non-pigmented morphology on all the LJ media prepared with extracts though with varied rate compared to the control. However, there was no growth on the media with standard drug and the media with distilled water as expected.

Conclusion: It can therefore be inferred from the result that aqueous and ethanolic extracts from the fruit skin and leaf of A. muricata at tested concentrations have no antimycobacterial activity.
Prevalence of culture-positive mycobacteria among suspected cases of pulmonary tuberculosis in Ahmadu Bello University Teaching Hospital, Zaria, Northern, Nigeria

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Background: Tuberculosis (TB) is a major cause of death and disability globally. Microscopy of Acid Fast Bacilli (AFB) is the routine method of diagnosis of tuberculosis in many developing countries and although faster and cheaper, requires a high bacterial load to obtain a positive result. The advent of Gen-Xpert has revolutionized diagnosis of tuberculosis though Mycobacteria other than tuberculosis (MOTT) are not often captured by this technology. The Gene-Xpert cannot also be used for follow up. Therefore culture still remains the gold standard. This research set out to determine the prevalence of culture positive Mycobacteria among suspected cases of pulmonary tuberculosis in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State, Nigeria

Methods & Materials: Patients aged ten years and above presenting with clinical features suggestive of pulmonary tuberculosis at ABUTH were recruited for this descriptive study. Three sputum samples were collected from each patient for Ziehl Neelsen staining and one early morning sputum from each patient was inoculated on Lowenstein Jensen media. Isolated Mycobacteria were identified as MOTT or Mycobacterium tuberculosis complex using immunochromatographic method. HIV status was also determined. A structured questionnaire was used to obtain demographic details of the patients. Descriptive statistics are presented.

Results: Of the total Mycobacteria isolated, 17(65.4%) were Mycobacterium tuberculosis complex (MTBC) while 9(34.6%) were Mycobacteria other than tuberculosis (MOTT). Out of 270 suspected pulmonary tuberculosis patients enrolled, 127(40%) were male and 60 (22.2%) were HIV positive. AFB microscopy was positive for 19 (7%) smears while 26 (9.6%) were culture-positive. Fifteen (11.8%) males and 3 (5%) of the HIV patients were also culture-positive

Conclusion: The need for culture in the diagnosis of pulmonary tuberculosis is important as there was obvious difference in prevalence of Mycobacteria detected by culture versus microscopy in this study, though not significant. Occurrence of MOTT among suspected TB cases further corroborates the urgent need for culture in our facilities.
Vitamin D deficiency, CNS inflammation, and clinical outcome in tubercular meningitis

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Background: Tubercular (TB) meningitis results in high mortality (about 40%) and neurological sequelae despite early treatment with anti-TB drugs and dexamethasone. Hence, adjunctive treatments are needed to improve the outcome. Vitamin D deficiency is associated with poor treatment outcomes in pulmonary TB. But, its effect in patients with TB meningitis is unknown. We tested the hypothesis that low serum 25-OH vitamin D levels would be associated with poor clinical outcome in patients with TB meningitis.

Methods & Materials: We prospectively studied 40 consecutive HIV-negative patients aged >12 years with TB meningitis (based on the international consensus criteria) up to treatment completion, death, or a minimum of 3 months. We estimated serum 25-OH vitamin D and IL-1β in the CSF by ELISA on pre-treatment specimens. We defined poor outcome as death or severe neurological sequelae (modified Rankin score of 3-6). Outcome was independently scored by two investigators before estimating vitamin D and IL-1β levels.

Results: Mean age of the patients was 38±13 years; 28 (70%) were men. Median (IQR) duration of symptoms was 20 (14-34) days. Twenty-two (55%) patients had grade 3, 12 (30%) had grade 2, and the remaining 6 (15%) had grade 1 TB meningitis. On follow-up, 21 patients had a poor outcome - 15 patients died; and 6 of the 25 survivors had severe neurological sequelae. Lower Glasgow coma score (9 [7-10] vs. 12 [10-15]; P = 0.007) was significantly associated with poor outcome. Twenty-two (55%) patients had deficient (<20 ng/mL; n = 10) or insufficient (20-30 ng/mL; n = 12) serum vitamin D levels. But, serum vitamin D level was not associated with clinical outcome (good vs. poor outcome: 28.30±14.96 vs. 35.92±17.11 ng/mL; P = 0.141). Further, serum vitamin D level did not correlate with CSF IL-1β level (Spearman’s rho = 0.083; P = 0.609).

Conclusion: Vitamin D deficiency/insufficiency is common among adults with TB meningitis. But, the low vitamin D levels are not associated with IL-1β, a marker of CNS inflammation, and clinical outcome. Hence, vitamin D supplementation may not be useful as an adjunctive treatment in TB meningitis.
The acceptability and feasibility of chemical prophylaxis for schoolchildren and adolescents with latent tuberculosis infection in Shanghai, China: A qualitative study

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Background: China has the 2nd highest burden of tuberculosis (TB). More about 40-45% of population were infected with TB and most of them were latent tuberculosis infection (LTBI). However, with better socioeconomic status, the prevalence of LTBI in Shanghai is relatively lower, especially among children. The contact history with TB patients would increase the risk of LTBI, and about 20% children contacts had a positive T-SPOT.TB result in shanghai. Children with LTBI could contribute to the pool of individuals with LTBI from which future active TB cases will arise. Chemical prophylaxis for people with LTBI is recommended by WHO to end TB occurrence. This study aimed to assess the acceptability and feasibility of chemoprophylaxis for schoolchildren and adolescents with LTBI in Shanghai, China.

Methods & Materials: Seven Focus Group Discussions (FGDs) were conducted in three districts in Shanghai among August and October, 2015. Forty-two participants including 15 TB contacts and 27 health care providers (either were TB program officials or general practitioners from CDCs and community health-centers) were invited. The Data about TB management, children TB-screening and acceptability of chemoprophylaxis for children with LTBI were collected by FGDs. Nvivo 10.0 was used to identify the key issues from these interviews through coding, categorization and grouping into emergent themes.

Results: While many children used to reject TB-screening due to concerns of radiation and haemospasia, the screening among children TB contacts were more acceptable by parents. Poor knowledge about positive T-SPOT.TB results and chemoprophylaxis has made it difficult to get the permission on prophylaxis for schoolchildren and adolescents with LTBI. Health providers could understand the potential benefits about chemoprophylaxis but still thought it unfeasible by now considering the adverse drug reaction, high costs for medication, long duration and unclear effect indicators. In addition, heavy workloads and poor incentive mechanisms were not uncommon in the basic TB control management.

Conclusion: The connection between households, schools, communities and hospitals should be established for further surveillance of adverse drug-reaction and health education on LTBI and chemoprophylaxis need to be strengthened. The current financing and incentive mechanisms of TB control need to be improved for better performance in TB control.
Effects of motivational interviewing on the treatment adherence of Tuberculosis patients
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Background: Tuberculosis is one of the deadly diseases worldwide (Enarson, 2000). The WHO reported that one third (1/3) of the world’s population is infected with TB. It is curable; however, if left untreated may be fatal. Non-adherence is one of the barriers in eliminating TB. Adherence declines due to lack of motivation of patients to complete their treatment (Pagulayan, 2008). Motivation affects self-efficacy of patients to adhere to their treatment (Treasure, 2004). Hence, modification on the attitude and behavior of patients may enhance treatment adherence (Dela Cruz, 2002). MI has been used in smoking cessation and substance abuse, however, no local literature has been found on its use for TB treatment adherence, and application in community settings. This study aims is to evaluate the effects of a nurse delivered MI as adjunct to standard health education to enhance treatment adherence of Tuberculosis patients in the health center.

Methods & Materials: The study utilized a true experiment, pre-post test design. Thirty Filipino newly diagnosed patients receiving treatment in the health center were randomly assigned to control and experimental groups using multistage cluster sampling. The experimental group received four (4) sessions of 30-minutes nurse delivered adjunct MI every week for one month, while the control group received standard health education. MI is a counseling style that used specific questions to direct behavior change by expressing empathy, developing discrepancy, rolling with resistance and supporting self-efficacy. Adherence was measured using Medication Adherence Self-Efficacy Scale and Sputum AFB microscopy before and 2 weeks after the intervention. A panel of experts reviewed the questionnaire to ensure validity, and the instrument to internal consistency.

Results: Knowledge about the disease and its treatment combined with motivation can increase self-efficacy to treatment adherence (Ngamvitroj, 2007). MI as adjunct to health education can increase treatment adherence (Riekart, 2011). Consequently, a rapid decrease in the number of M. tuberculosis in sputum. Moreover, studies have shown that MI has significant psychological (75%) and physiological (72%) effect to diseases (Rubak, 2005).

Conclusion: Motivational Interviewing delivered by the nurse as an adjunct to the standard health education is effective in enhancing treatment adherence of PTB patients in the health center.
The referral pathway of presumptive drug resistant tuberculosis in the urban poor areas of Metro Manila, Philippines  

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**Background:** The National Tuberculosis Control Program (NTP) guidelines emphasize that all presumptive Drug Resistant Tuberculosis (DRTB) with history of previous tuberculosis (TB) treatment and with Multi-Drug Resistant Tuberculosis (MDRTB) contacts should be screened for MDR. Due to lack of tools to identify the referral outcome of presumptive DRTB, the RJPI developed an MDRTB Presumptive Masterlist to account referrals from health center to MDR treatment center. The study aims to understand gaps in the referral pathway experienced by Local Government Units (LGUs) and Non-Government Organizations (NGOs) from initial consultation until initiation of treatment.

**Methods & Materials:** A retrospective descriptive study of patients’ data registered on MDRTB Presumptive Masterlist of eighteen Directly Observed Treatment Short-Course (DOTS) facilities in District 1 Tondo, Manila and Payatas, Quezon City from October 2012 to September 2013, were reviewed and analyzed using structured questionnaire. Unpaired t-test used in comparing the turnaround time between LGUs and NGOs including time between Direct Sputum Smear Microscopy (DSSM) results presented to patient and referred to MDR treatment center. A p-value <0.05 was considered statistically significant. All analysis performed using EZR with graphical user interface for R.

**Results:** A total of 378 Presumptive DRTB was identified and listed in the masterlist. Among them, 97% (368/378) referred and 90% (333/368) screened at MDR Treatment center. Among screened, 85% (283/333) completed the process of MDR screening and provided with an appropriate treatment based on NTP guidelines. 9.5% (35/368) were not screened mainly due to lost to follow up. The duration of time between sample collected and examined at laboratory of NGOs was significantly longer than LGUs (n=283; p<0.001). The time duration between the release of DSSM results and presentation of patient at NGOs was significantly shorter than LGUs (p=0.009).

**Conclusion:** Development of MDRTB Presumptive Masterlist has facilitated tracking of patients due for diagnosis and treatment. Referral system between health center and MDR treatment centers should be strengthened for proper patient endorsement and provided with an appropriate action. The NGOs should lessen diagnosis delays and LGUs should follow up patient for early start of TB treatment.
Predictors, outcome, profile of anti-tubercular drug induced hepatitis – A prospective nested case - control study in a South Indian tertiary hospital

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Background: Tuberculosis (TB) remains a major global health problem. The first line anti-tubercular drugs are hepatotoxic. Despite adequate knowledge, we are still unable to predict anti-tubercular drug induced liver injury (DILI) before initiation of treatment.

Methods & Materials: This case-control study was nested in a cohort of patients from Christian Medical College, Vellore who were newly diagnosed to have tuberculosis and started on treatment. It was carried out from April 2013 to May 2014. All patients who present with suspected ATT related hepatotoxicity were also enrolled in the study. All patients on treatment were clinically assessed for symptoms of hepatitis at every visit until completion of treatment. The risk factors for ATT induced hepatitis were identified by bivariate analysis and logistic regression analysis with odds ratio and 95% confidence interval.

Results: A total of 393 patients were eligible for our study which included 5 patients presenting with DILI. Patients on DOTS regimen had lower rates of HIV infection and disseminated disease but had greater under nutrition when compared with patients on daily regimen. 43 patients out of 393 patients developed DILI. The incidence of anti-tubercular drug induced liver injury was 9.7 % (95% C.I 7-13.2%) with lower incidence among patients on DOTS regimen (14% Vs 3.5%). HIV infection, daily regimen, disseminated disease, hypoalbuminemia and chronic liver disease were independent risk factors for development of DILI. A prediction score of > 5 based on the above risk factors will predict DILI with a sensitivity and specificity of 74% and 67% respectively. All cause mortality in DILI was 4.7 % (2 patients). 36 patients (84%) had complete resolution of hepatitis. Rechallenge by both ATS and BTS guidelines had similar successful rechallenge rates.

Conclusion: The incidence of anti-tubercular DILI was 9.7 %.The study suggests that the combination of risk factors of extensive TB disease, HIV infection and undernutrition increase the vulnerability to DILI particularly with daily TB treatment regimen, emphasizing the role of acquired risk factors in the development of DILI. The predictive scoring system proposed from our study needs to be validated by a well designed prospective study.
Thyroid tuberculosis: report of a case and review of literature
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**Background:** thyroid tuberculosis is a rare disease, this is the probably the polymorphic clinical presentation and confusing with other thyroid diseases including cancer and thyroid hemorrhagic cysts. Ultrasound is currently a diagnostic and monitoring means, and treatment based on the use of anti-TB first to follow a non-treatment of associated endocrinopathy. Its diagnosis is often delayed, responsible for a significant morbidity and mortality. The objective of the study was to describe the clinical, radiological signs that should prompt clinicians to watch for the disease.

**Methods & Materials:** this is a young man of 38 years without medical history including no history of pulmonary or extrapulmonary tuberculosis or TB contagion, admitted to Department of Infectious and Tropical Diseases in support of a retroviral infection confirmed HIV1 revealed by prolonged fever and cervical lymphadenopathy associated with poly encrypted to 12kg weight loss in two months, the clinical picture worsened by the appearance of headache and dizziness.

**Results:** the diagnosis was retained before the removal of casein and AFB to FNA of cervical lymphadenopathy fine needle and suspicion on the location thyroidienne de tuberculosis before the tremor of the extremities, and cervical ultrasound is made objectifying an echo heterogeneous structure thyroid nodules seat 3, heterogeneous hypoechoic with multiple bilateral carotid jugular lymph nodes. Osteoarticular realized before pancytopenia biopsy shows tuberculoid granuloma with caseous necrosis and diagnosis of tuberculous multifocal gonglionnaire location and hématopoëtique confirmed, thyroid and lung porobable is retained.

An assay was 0.58mUI TSHus / l (normal rate), but the dosage of T3et T4 was not made fault of lack of means. The antibacillaire treatment is started according to the protocol 2RHZE / 4RH with good clinical course.

**Conclusion:** Thyroid tuberculosis is a rare entity but should be considered in the differential diagnosis of cervical masses.

FNA or fine-needle biopsy guided diagnostic procedures are safe and inexpensive, that can prevent the use of unnecessary thyroidectomy.
Trend of multidrug resistance extra pulmonary tuberculosis cases presenting to a tertiary care hospitals in Northern part of India

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Background: The emergence and spread of multidrug-resistant tuberculosis (MDR-TB) is a major public health problem in India. Extra pulmonary tuberculosis (EPTB) among MDR-TB is contributing to the burden of disease and does not receive specific attention in international control strategies. The aim of this study is to investigate trends and patterns of MDR-TB from clinical isolates from EPTB cases in Northern India.

Methods & Materials: A total of 1206 specimens were processed from patients suspected of having EPTB with varied presentation. Specimens were processed by Ziehl Neelson staining, BacT/ALERT 3D culture, identification of *Mycobacterium tuberculosis* complex (MTBC) by IS6110-PCR. First line drug susceptibility testing was performed by 1% proportional method by BacT/ALERT 3D system. MDR-TB isolates were further characterized by GenoType® MTBDRplus assay.

Results: Specimens from 260 (21.5%) cases were culture positive for mycobacteria. Of these 192 (73.8%) were *M. tuberculosis* complex isolates. Of these 78 (41.6%) strains were resistance to one or more antitubercular drug. MDR-TB resistance by phenotypic method was obtained in 28 (14.5%) cases. However 28 (14.5%) strains were confirmed MDR-TB by genotypic method. The most prominent mutations in *rpoB*, *katG* and *inhA* genes were 78% in S531L, 95% in S315T1, and 21% in C15T region respectively (p <0.05).

Conclusion: The high prevalence of MDR-TB among EPTB cases is obtained in this region of India. 78% in S531L and 95% in S315T1 were most prominent mutation patterns in MDR-TB cases. Early recognition of MDR strain by molecular method can help in minimizing the risk of further resistance and limits spread of drug-resistant strains.
Evaluation of the diagnostic performance of MTBDRplus VER 2.0 line probe assay for the detection of MDR-TB in sputum samples referred to National TB Reference Laboratory, Ethiopian Public Health Institute 

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**Background:** Multi drug resistant tuberculosis(MDR-TB) is more difficult to diagnose and treat, leading to high mortality. Accurate and rapid detection of MDR-TB is critical for timely initiation of treatment. Evaluating new drug resistance diagnostic tools such as Genotype MTBDRplus VER 2.0 assay offer opportunity to scale up drug susceptibility testing( DST) capacity in Ethiopia.

**Methods & Materials:** A cross sectional study was conducted from December to August, 2015 on presumptive MDR-TB patients. Analysis of 72 smear positive and 197 smear negative sputum samples was done with Genotype MTBDRplus VER 2.0 assay and compared with the reference, BACTEC MGIT 960 culture and DST. Sensitivity, specificity, PPV and NPV of the MTBDRplus VER 2.0 assay was calculated, comparing the results with the reference method and results was interpreted based on 95% confidence interval, statistical significant was taken at p-value <0.05.

**Results:** The sensitivity, specificity, PPV and NPV of Genotype MTBDRplus VER 2.0 assay were 96.4, 100, 100 and 96.9%, respectively for the detection of MDR-TB from direct smear positive sputum samples. Only 14(54%) samples had valid results with LPA among the 26 smear negative culture positive samples. The remaining 8(30.6%) and 4(15.4%) were invalid and negative with LPA, respectively. The sensitivity and specificity of Genotype MTBDRplus VER 2.0 assay was 100% for the detection of MDR-TB among 14 direct smear negative and culture positive sputum samples. The most common mutations associated with RMP and INH resistance was S531L and S315TL, respectively. A single rare mutation (C15T/A16G) was also detected in this study.

**Conclusion:** The diagnostic performance of Genotype MTBDRplus VER 2.0 assay in direct smear positive sputum sample was highly sensitive and specific for early detection of MDR-TB. However, the diagnostic performance of Genotype MTBDRplus VER 2.0 assay in direct smear negative sputum sample was low and showed high level of invalid results so it is unlikely to implement Genotype MTBDRplus VER 2.0 assay for the detection of MDR-TB in direct smear negative sample in our routine settings until the method is optimized. Hence, large scale further studies are needed in direct smear negative samples.
Insertion Sequence IS6110 mapping, a tool to characterise TB strains into genetic lineages
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Background: Tuberculosis (TB) along with HIV infection is the major cause of mortality worldwide including the 29 million people in Nepal. The steady rise in the number of Multi-drug Resistant (MDR) TB cases in the last few years has increased the challenges facing the scientists and health professionals alike. With no previous epidemiological data available on transmission patterns of *Mycobacterium tuberculosis* complex (MTBC) in Nepal, the focus of this study is to categorise the TB samples for the first time using IS6110 fluorescent amplified fragment length polymorphism (FAFLP) PCR into different genetic lineages.

Methods & Materials: The bacterial DNA from clinical isolates of 176 TB patients in Nepal along with the reference strain H37Rv were extracted using the CTAB method and subjected to FAFLP PCR, using four differentially labelled selective primers. The samples separated on the ABI Genetic Analyser 3730xl were then analysed using the PeakScanner software and were identified using their fluorescent tag. The 4-dye FAFLP data collected from the different profiles were later recorded in the BioNumerics software v6.1 and compared with the reference global collection of TB samples.

Method schematic of 4-dye IS6110 FAFLP PCR. In the example shown above (A), red fragment is generated as TaqI-C anneals to the C base in the DNA where base C is amplified and in example (B) exact insertion of IS6110 in the Mtb genome is identified using

Results: Out of 176 samples analysed, 64 samples belong to the Central Asian (CAS) lineage or principal genetic group 1(PGG1), 33 samples belong to the Beijing lineage (PGG1) and the rest of the samples belong to other genetic groups – LAM, Haarlem, X (PGG2) and T (PGG3). Also, all but two of the sixteen insertion sites of H37Rv were mapped using this technique.

UPGMA derived Dendrogram showing the distribution of the 176 bacterial DNA isolates with CAS and Beijing (shown in red) lineages (>50%) being the major principal genetic group in Nepal

Conclusion: From the data above, it is clear that 55% of the samples fall under the CAS and the Beijing group. This novel information on TB population in Nepal is geographically relevant as it is surrounded by China in the north (dominated by Beijing strains) and the other Central Asian countries in the south (dominated by CAS strains). As the prevalence of TB infection including the MDR types is high in the Nepalese population, the 4-dye FAFLP typing technique will not only aid the contact tracing of samples but also shows a picture on the predominant PGGs found in Nepal which can be helpful in future epidemiological surveillance or outbreaks.
Tuberculous osteomyelitis of mid-clavicle in a healthy normal girl

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**Background:** 16 year old girl with no known comorbidities presented with complaints of painful restriction of movement of left shoulder with no history of fever followed by development of a swelling over the left mid clavicular region. The swelling was painful with pain radiating to the left arm.

**Methods & Materials:** Case Report

**Results:** FnaC done frank pus was aspirated and was found to be teeming with gram positive cocci in singles chains and clusters. Pus culture grew Staphylococcus aureus sensitive to clindamycin and patient was initiated on the same.

Despite being on the appropriate antibiotic the swelling had not resolved and continued to be painful.

MRI left shoulder was done revealed destruction of medial aspect of clavicle with evidence of collection arising from the mid clavicular region extending along the pectoralis major muscle origin, posterior aspect of clavicle and posterior aspect of sternum.

Excision and biopsy of the left clavicle and drainage of abscess done. Histopathology revealed chronic granulation tissue with foreign body reaction. TB PCR sent was positive and sensitive to Rifampicin; TB culture done grew mycobacterium tuberculosis.

**Conclusion:** 16 year old girl with no comorbidities had a Tuberculous cold abscess arising from the mid clavicular region which was secondarily infected with Staphylococcus aureus. Patient initiated on 4 drug antitubercular treatment is on regular follow up.
A study on factors influencing management and outcomes of tuberculosis

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**Background:** Tuberculosis is a matter of Public Health remains a devastating disease throughout the world. The fact that it remains the eighth leading cause of death in the world speaks to the challenges facing practitioners and public health officials as they try to control a disease. Education and awareness regarding TB is essential along with adequate TB management, which helps to decrease morbidity and mortality associated with it.

**Methods & Materials:** To determine the factors influencing management and outcomes of tuberculosis, dosage calculation of drug, medication adherence, adverse effects. Dosing based on body weight or body surface area assumes that drug pharmacokinetic parameters increase in proportion with increasing body size. The conventional weight based dosing strategies are more likely to result in drug overexposure or underexposure among patients. Alternate weight descriptors such as ideal body weight, adjusted body weight, are used to prevent them.

Single center, Prospective Observational study was done in Apollo hospitals. Study was conducted by selecting patients who were suspected or diagnosed with Tuberculosis. Data collection was done from Inpatient files, Outpatient prescriptions, Medical Records Departments, Microbiological Department, by Patient interviews (both direct and by phone calls) for Morisky adherence scale after getting the Informed Consent Form. Data analysis was done by correlating one parameter with another and the data was presented in form of tables and charts.

**Results:** Among the 67 subjects enrolled for the study, 57 were analyzed. Among which 34 (59.6%) were males. Higher percentage of TB incidence was seen in the age group of 18-30 years (29.8%). Standard regimen (HRZE) was used in 46 (80.2%) of the cases. Overall 24 Adverse Drug reactions were observed, in which 11 patients experienced increase in uric acid levels/joint pains followed by increase in LFTs.

**Conclusion:** Our study revealed Adherence was high in patients who had better understanding about their disease. Alternate weight descriptors such as ideal body weight, adjusted body weight, are used to prevent drug overexposure and underexposure, thus by preventing adverse effects and ineffective treatment.
Under-recognised and misdiagnosed; post surgical rapidly growing mycobacterial infections in South India

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Background: Rapidly growing mycobacteria (RGM) are environmental organisms that can cause post-operative wound infections. Infections typically occur after laparoscopic surgery due to inadequate sterilization of heat-sensitive instruments. We describe the clinical presentation and management of post-operative RGM infections at Christian Medical College (CMC), a large tertiary referral hospital in South India.

Methods & Materials: Laboratory records from 1st January 2012 to 31st August 2015 were examined to identify patients with culture positive post-operative RGM infections. The electronic medical records of these patients were reviewed together with their haematological, histological and radiographic data.

Results: Over this period, 32 patients were diagnosed with culture proven RGM infection as a consequence of surgery. *Mycobacterium fortuitum* was the commonest isolate (46.9%), followed by *M. abscessus* (31.2%) and *M. chelonae* (18.8%). Most patients had wound infections (96.9%); 78.1% extended into underlying muscle and 28.1% into structures deep to muscle. 37.5% patients had infection associated with prosthetic material including surgical mesh, pacemakers, cardiac valve and a neurosurgical shunt. Surprisingly, most patients (65.6%) had undergone open surgical rather than laparoscopic procedure (25%).

Only 4 patients (12.5%) acquired RGM infection following surgery at CMC. Over this period, 96,713 operations were performed resulting in an infection rate of 0.004%. 87.5% patients underwent operation at a different hospital, presenting to CMC a median 4 months after operation. 43.8% received inappropriate treatment for wound infection before presenting to CMC. 37.5% received antibiotics and 9.4% empirical antitubercular therapy, highlighting poor knowledge about RGM infections.

All patients were treated with surgical debridement; 75% received subsequent antibiotics consisting of a two or three drug combination of amikacin, levofloxacin/moxifloxacin, clarithromycin or linezolid. Patients jointly managed by surgeons and infectious disease physicians had a higher rate of clinical response (75%) with less loss to follow up (25%) than those managed exclusively by surgeons (43.8% and 57.25% respectively).

Conclusion: RGM infections continue to complicate routine operations in India, although they are a rare complication of surgery in our hospital. They are under-recognised and frequently misdiagnosed resulting in delays in appropriate treatment. Higher clinical response rates are seen where management involves surgeons and infectious disease clinicians with laboratory support from microbiologists.
Interferon Gamma Release Assay (IGRA) friend or foe - Clinical application of IGRA in a tuberculosis endemic country
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Background: Though IGRA has been compared to tuberculin skin test, there is scarce clinical data in India regarding correlation of IGRA for diagnosis of active or latent tuberculosis. The role of IGRA for diagnosis of latent tuberculosis and initiation of prophylaxis in an endemic country has often been questioned.

Methods & Materials: All adult patients (age>18 years) for whom an IGRA test was done as part of their clinical work up in our center, in 2013 were included. Demographic and clinical details including underlying diagnosis, indications, laboratory investigations (Mycobacterial smears, cultures and Xpert MTb/rif), initiation of prophylaxis or development of tuberculosis within 1 year of follow up were recorded.

Results: A total of 434 patients were included, the majority were males (61%) and the mean age was 39.4 years. The common indications for ordering IGRA was to rule out active tuberculosis in 329/434(75.8%) and for diagnosis of latent tuberculosis prior to initiation of prophylaxis in 68(15.7%). IGRA was negative in the majority of the patients (63.6%). Among the IGRA positive 158/434(36.4%) only 4 were initiated on prophylaxis for possible latent tuberculosis whereas in the IGRA negative none received prophylaxis. In the IGRA positive 50(32%) and in the IGRA negative 47(17.6%) received empirical antituberculosis therapy for suspected tuberculosis. M. tuberculosis was however confirmed by cultures and/or PCR in 14(3.2%) of which 8 were initially IGRA positive and 6 were IGRA negative. In the sub group where IGRA was used to diagnose latent tuberculosis- 42/53(79%) in the IGRA negative and 14/15(93%) among the IGRA positive were on immunosuppressive drugs. Of these though 15 patients were IGRA positive, only 2 (13.3%) initiated prophylaxis possibly due to high level of Isoniazid monoresistance in our hospital.

Conclusion: A majority of the patients were found to be IGRA negative, which was a surprising finding in a high tuberculosis burden country. IGRA is still being used as a supporting tool for a diagnosis of active tuberculosis in the absence of other confirmatory microbiological evidence. Even if IGRA is used to rule out latent tuberculosis there is a reluctance to initiate prophylaxis.
Outcomes of multidrug resistant tuberculosis treatment among human immunodeficiency virus co-infected patients taking anti-retroviral treatment at Sizwe Tropical Disease Hospital Johannesburg, South Africa
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Background: Multidrug resistant-tuberculosis (MDR-TB) is a threat to global tuberculosis control which is worsened by human immune-deficiency virus (HIV) co-infection. There is however paucity of data on the effects of antiretroviral treatment (ART) before or after starting MDR-TB treatment. This study determined predictors of mortality and treatment failure among MDR-TB-HIV co-infected patients on ART.

Methods & Materials: A retrospective medical record review of 1200 HIV co-infected MDR-TB patients admitted at Sizwe Tropical Disease Hospital, Johannesburg from 2007 to 2010 was performed. Chi-square test was used to determine treatment outcomes in MDR-TB-HIV co-infected patients on ART. Multivariable logistic regression and Poisson models were used to determine predictors of mortality and treatment failure respectively.

Results: Mortality was higher (21.8% vs. 15.4%) among patients who started ART before initiating MDR-TB treatment (p=0.013). Factors significantly associated with mortality included: the use of ART before starting MDR-TB treatment (OR 1.65, 95% CI 1.002-2.73), severely-underweight (OR 3.71, 95% CI 1.89-7.29) and underweight (OR 2.35, 95% CI 1.30-4.26), cavities on chest x-rays at baseline (OR 1.76, 95% CI 1.08-2.94), presence of other opportunistic infections (OR 1.80, 95% CI 1.10-2.94) and presence of other co-morbidities (OR 2.26, 95% CI 1.20-4.21). Factors predicting failure were severe anaemia (IRR (OR 4.72, 95% CI 1.47-15), other co-morbidities (OR 2.39, 95% CI 1.05-5.43) and individualised regimen at baseline (OR 2.15 95% CI 0.98-4.71).

Conclusion: High mortality among patients already on ART before initiating MDR-TB treatment is a worrisome development. Management of adverse-events, opportunistic infections and co-morbidities in these patients is important if the protective benefits of being on ART are to be maximized. There is the need to intensify intervention programmes targeted at early identification of MDR-TB, treatment initiation, drug monitoring and increasing adherence among HIV co-infected MDR-TB patients.
Cellular iron status affects drug susceptibilities and biofilm formation of mycobacterium

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Background: Continuous and widespread usage of antitubercular drugs leading Multi-Drug Resistance (MDR) acquired by Mycobacterium tuberculosis (MTB) demands immediate search for novel targets and mechanisms. The ability of MTB to adapt the hostile environment is essential for its survival and confers the basis of successful infection. A crucial condition that MTB must overcome during the establishment of infection within the host is iron limitation, since iron not freely available is required by both bacteria and humans. This study aims to investigate the effect of iron deprivation on drug susceptibilities of known anti-TB drugs and biofilm formation in Mycobacterium smegmatis, a “surrogate of MTB.”

Methods & Materials: Drug susceptibility was tested using broth microdilution to determine minimal inhibitory concentration (MIC) and spot assays under iron deprivation. Membrane permeability and passive diffusion of drugs were assessed by nitrocefin hydrolysis and EtBr efflux assays respectively. Membrane disruption was also studied with transmission electron microscopy (TEM). Biofilm formation was quantitatively measured with crystal violet (CV) dye binding.

Results: The study revealed that iron deprivation led to enhanced potency of the most commonly used first line anti-TB drugs that could be reverted upon iron supplementation. It was explored that membrane homeostasis is disrupted upon iron deprivation as revealed by enhanced membrane permeability, hypersensitivity to membrane perturbing agent leading to increased passive diffusion of drug and TEM images showing detectable differences in cell envelope architecture. It was also shown that hypoxia but not alkaline pH which mimics iron deprivation also leads to enhanced potency of anti-TB drugs. Furthermore, iron seems to be indispensable to sustain genotoxic stress suggesting its possible role in DNA repair machinery. Finally, iron deprivation also inhibits biofilm formation which is an important virulence attribute.

Conclusion: The study for the first time established a link between cellular iron, drug susceptibility and biofilm of Mycobacterium suggesting iron as novel determinant to combat MDR.
TRUNCATE-TB: an innovative trial design for drug-sensitive tuberculosis
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**Background:** The number of potential regimens of drug treatment for TB is vast, meaning that evaluating each new treatment against a control in separate two-arm trials requires a huge amount of resources. There is, therefore, a need for innovative trial designs that can evaluate drug regimens simultaneously.

**Methods & Materials:** TRUNCATE-TB (two-month regimens using novel combinations to augment treatment effectiveness for drug-sensitive tuberculosis) is a randomised, open-label, multi-arm, multi-stage (MAMS), parallel group strategy trial. The MAMS design has been applied successfully in a trial of multiple regimens for prostate cancer, and more recently in a TB trial. In the MAMS design, the trial starts with multiple arms, and, as it progresses, recruitment to those arms that do not show sufficient promise on an early interim outcome measure are discontinued, whilst recruitment to the control arm and remaining promising novel boosted arms continues until sufficient numbers of patients have been enrolled to assess the outcome on the defined primary outcome. Recommendations about stopping or continuing arms are made by an Independent Data Monitoring Committee on the basis of safety and efficacy data.

**Results:** Up to 1080 adult patients with pulmonary TB (diagnosed using sputum GeneXpert) will be randomised at equal probability to receive 6-months standard treatment as a control, or to one of a number of boosted regimens (these regimens are combinations of standard drugs with new or repurposed drugs including: high-dose rifampicin, linezolid, clofazimine, bedaquiline, delamanid, rifapentine or levofloxacin.

**Conclusion:** The primary aim of the trial is to determine whether a strategy of treating drug-sensitive TB for 2 months with one of a number of novel combination regimens and re-treating relapse with a 6-month course of standard treatment will be non-inferior to the standard WHO-recommended 6-month treatment/8-month retreatment approach in terms of TB sputum culture status at 2 years after randomisation. The secondary aims are to assess the possible advantages of the TRUNCATE-TB management strategy compared to the standard management strategy from the patient perspective (including acceptability, quality of life and clinical adverse events) and the programme perspective (including treatment adherence, default, new drug resistance and community transmission) as well as cost-effectiveness.
Identification of new efflux pump proteins from multidrug resistant Mycobacterium tuberculosis and screening for peptide based efflux pump inhibitors.

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**Background:** Tuberculosis (TB) continues to be one of the main causes of morbidity and mortality worldwide. The alarming increase in the rate of HIV-related TB, pediatric TB, latent TB, Multi Drug Resistant (MDR) and Extensively Drug Resistant (XDR) TB pose serious problem around the world. The treatment for tuberculosis requires very long duration with combination of antibiotics (6-8 months for new cases and 18-24 months for MDR TB). Two mechanisms are thought to be involved in the natural drug resistance of mycobacteria: the mycobacterial cell wall permeability barrier and active multidrug efflux pumps. Genes encoding drug efflux transporters have been isolated from several mycobacterial species. These proteins transport tetracycline, fluoroquinolones, aminoglycosides and other compounds. Recent reports have suggested that efflux pumps may also be involved in transporting isoniazid, one of the main drugs used to treat tuberculosis. In most bacterial species, efflux pump inhibitors (EPI) can reduce resistance levels both in wild-type strains and in those with acquired target mutations.

**Methods & Materials:** Identification of MTB efflux pump genes
- MDR-MTB identification
- Gene Cloning from MDR-MTB, Nucleotide sequencing and Mutation analysis
- Isolation and identification of Peptide inhibitors from Lactic acid bacteria (LAB)
- Mycobacterium tuberculosis inhibitor assay
- Luciferase assay

**Results:** In this study we have identified a list of genes which might function as potential drug efflux pumps from the MDR-MTB clinical isolates. These genes were identified based on sequence analysis of the corresponding efflux genes with that of the MTB strain H37Rv. Crucial mutations in these genes might have an important role in the drug efflux in MDR-MTB. The identified genes were expressed in drug resistant E.coli. The isolated peptides from LAB and known drug efflux pump inhibitors in combination with RIF and INH were tested in E.coli (Efflux genes) and MDR-MTB.

**Conclusion:** Nucleotide comparison studies with the H37Rv strain we have identified potential candidate genes related to drug efflux in MDR-MTB strains. Novel peptide inhibitors identified from Lactic Acid Bacteria shows high inhibition against the MDR-MTB. These peptides has the potential to be developed as efflux inhibitors against MDR MTB.
Determinants of MDR-TB in a district tuberculosis centre of a metropolitan city: A case - control study

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Background: Multidrug resistant tuberculosis (MDR-TB) is of growing health concern globally and in India. MDR-TB is defined as Mycobacterium tuberculosis resistant to Isoniazid and Rifampicin. The current prevalence of MDR-TB in India is 2.3% (ranging from 1.8%-2.8%) and 17.2% (ranging from 14.9%-19.5%) among newly diagnosed and previously treated cases respectively. Increased cost, more side effects and longer duration of treatment makes MDR-TB management a great challenge.

Methods & Materials: The present study was conducted as an Unmatched Case Control study to identify the determinants of MDR-TB. The study was conducted in all the 14 Tuberculosis units (TU) of Revised National Tuberculosis Control Programme (RNTCP) under the Municipal Corporation of Bengaluru. The source of the study subjects were patients registered under 14 TUs during the period of January 2013 to June 2014, with 52 MDR-TB patients as Cases and 53 non MDR-TB patients as Controls. Cases and controls were confirmed by culture and drug susceptibility test done at the Intermediate Reference Laboratory (IRL), Bengaluru. Both cases and controls were interviewed at their residence using a pretested semi structured questionnaire.

Results: Univariate analysis including descriptive statistics along with Chi-square test and multivariate logistic regression were used to analyse the data. The mean age of the cases was 38.5±15.8 years which was almost similar to that of the controls (38.5±13.0 years). Univariate analysis revealed - the number of previous episodes of TB, type of health setup approached during the first episode of TB, treatment outcome of the first episode of TB, gender, employment status, religion, education and socioeconomic status as statistically significant determinants of MDR-TB. After adjusting for potential confounders with multivariate logistic regression – the number of previous episodes of TB (OR= 4.08, 95%CI: 1.53-10.86), the treatment outcome of the first episode of TB (OR= 2.15, 95%CI: 1.42-3.25) and gender (OR=3.14, 95%CI: 1.18-8.29) were found to be independent determinants of MDR-TB.

Conclusion: Hence prompt treatment of newly diagnosed cases of tuberculosis in the first instance is critical in prevention of MDR-TB. Adequate counselling regarding treatment adherence to the patients at the first episode of TB will ensure compliance and reduce the chances of drug resistance.
Mycobacterial dormancy associated proteins: Role in the survival of bacteria under stress conditions

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Background: Mycobacterium tuberculosis, the cause of tuberculosis in humans, is present approximately in one third of the world’s population, mostly in a dormant state. The proteins encoded by the dormancy survival regulon (DosR regulon) are mainly responsible for survival of the bacilli in a latent form. To maintain latency, mycobacteria orchestrate a balanced interplay of different cytokines secreted by immune cells during the granulomatous stage. The function of most of the DosR regulon proteins of M. tuberculosis is unknown. *Rv3131* and *Rv2004c* are members of DoSR antigens category and conserved hypothetical proteins which are significantly up-regulated under different stress conditions. *Rv3131* contains FMN binding nitroreductase domain and is predicted to be responsible for virulence, detoxification and adaptation of bacteria under hostile conditions. *Rv2004c* was reported to be localized on the surface of M.tbc and its high active binding peptide sequence interacts with U937 macrophages and A549 epithelial cells.

Methods & Materials: *Rv3131* and *Rv2004c* genes were cloned and respective proteins were purified.

Results: In this study, we have shown that the DosR regulon proteins, encoded by the genes *Rv3131* and *Rv2004c*, can stimulate macrophages and peripheral blood mononuclear cells (PBMC) to secrete important cytokines that may be significant in granuloma formation and its maintenance. Computational modeling, docking and simulation study suggested that these proteins might interact with TLR2. This was further confirmed through the interaction of recombinant *Rv3131* and 2004c with TLR2 expressed by HEK293 cells. In THP-1 cells treated with *Rv3131* and 2004c, Tlr2 mRNA levels were significantly increased. When in vitro differentiated THP-1 cells were treated with recombinant *Rv3131* and *Rv2004c*, increased secretion of TNF-α, IL-1β and IL-8 was observed in a dose dependent manner. Similarly, human PBMCs when treated with recombinant *Rv3131* and 2004c, showed upregulated secretion of pro-inflammatory cytokines IFN-γ, TNF-α, IL-1β and IL-8. FACS analyses on THP-1 cell surface following *Rv3131* and *Rv2004c* protein treatment demonstrated increased expression of TLR2. Further experiments are being carried out to decipher the functional role of these proteins.

Conclusion: The cytokine profiles dissected herein point to a possible role of these proteins in maintenance of latency with the help of the pro-inflammatory responses.
Validation of non-uniform illumination correction techniques in microscopic digital TB images using image sharpness measures

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Background: Tuberculosis (TB) is a communicable disease for which an early diagnosis is essential to control the disease. The microscopy-based TB screening is the conventional method employed for TB identification in sputum smears. Fluorescence microscopy-based diagnosis provides improved sensitivity and benefits large number of TB burdened communities across the globe. Microscopic images are often corrupted by intensity variations because of inherent imperfections of the image formation process. This may result in false positives which is the potential shortcoming of fluorescence microscopy.

Methods & Materials: The fluorescence-stained slides were prepared at South African National Health Laboratory Services, Groote Schuur Hospital in Cape Town. The images (N=100) were captured using a camera in monochrome binning mode attached to a 20x objective fluorescence microscope of 0.5 numerical aperture. The camera (AxioCam HR) has a resolution of 4164 x 3120 with a pixel size 6.45 µm (h) x 6.45 µm (v).

The illumination correction methods adopted in this work include surface fitting method, multiple regression method and bidirectional empirical mode decomposition. The results of illumination correction are validated using the image sharpness measures. This includes derivative-based, statistical, histogram-based and transform-based parameters.

Results: It is observed from the results that surface polynomial fit-based correction performs better among the illumination correction techniques. The intensity profile of the corrected image reveals the performance of the method. Also, the most significant sharpness parameters-based on derivative, statistical, image histogram and transform showed the effectiveness of the method which could be suitable for the non-uniform illumination correction of these images. Results demonstrate that the seven most significant (p < 0.0001) sharpness parameters present distinct variation with the implementation of surface fitting illumination correction method. This proves the suitability and efficiency of the method for these images.

Conclusion: Although illumination correction is a long standing research topic and many algorithms have been proposed, the selection of the optimal algorithm for specific experimental microscopy applications remains ad hoc. The application of the surface fitting method is especially useful in pre-processing of digital sputum smear images, and could be used for reliable identification and classification of TB objects (bacilli and non-bacilli).
Paradoxical reaction (PR) in HIV negative patients with tuberculosis: Case series

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Background: PR, an entity with an incidence of 10-15%, is an exuberant inflammatory reaction resulting in clinical or radiological worsening after initiation of appropriate ATT in the absence of disease relapse, drug resistance or presence of another diagnosis. This is well described in the HIV infected as IRIS but with scarce data in the non-immunocompromised individuals. This is a case control study which elaborates on the incidence, risk factors and clinical outcomes of patients with paradoxical worsening in tuberculous lymphadenitis.

Methods & Materials: Between 2008-2012, 124 patients were seen in ID Clinic with a diagnosis of probable Tuberculous Lymphadenitis. HIV positive and proven MDR tuberculosis patients were excluded. 36 patients with PR were identified based on appearance of new nodes or enlargement of pre-existing nodes with fluctuation or discharge while on treatment. Univariate analysis was done to identify factors associated with PR.

Results: 52 were men with 13 in PR group and 72 were women with 23 in PR group. Mean baseline ESR was 48.4±7.03 which declined to 32.57±5.62 in PR group whereas in control group ESR declined from 54.6±4.51 to 38.08±5.88. Mean baseline CRP was 24.84±5.65 which declined to 14.23±5.29 in PR group whereas in control group CRP declined from 30.56±5.78 to 9.70±4.55. Mean baseline absolute lymphocyte count was 1588.59±121.65 in PR group and 1630.34±94.81 in control group. PR occurred as early as 2 months to 1 year after ATT. Radiological imaging done at the time of PR in 17 patients showed well defined nodes with hypo-echoic centre. Cultures were negative in 28 and 16 had positive AFB smears(scanty). 18 patients needed surgical excision and drainage, 5 and 3 patients were initiated on steroids and pentoxiphylline respectively.

Conclusion: In conclusion, PR must be considered when patients with tuberculosis on appropriate ATT presents with worsening symptoms. Low baseline absolute lymphocyte counts with a surge during PR along with rise in ESR and CRP are described in literature which however could not be elucidated in our analysis. We found that most of these patients during PR have scanty or negative smears with negative cultures along with sonographic evidence of hypo-echoic nodes.
Comparative analysis of the host mediated antigen-specific responses In Indian cohorts with different TB infected states

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Background: Tuberculosis (TB) remains one of the world’s deadliest communicable diseases. In 2014, 9.6 million people developed TB and 1.5 million died from the disease. Emergence of MDR or XDR strains of Mycobacterium tuberculosis (Mtcb), coupled with the lack of effective vaccines and sensitive diagnostic kits has severely compromised the control of global TB epidemic. Although, (~90%) infected subjects are able to contain infection in a sub-clinical dormant stage known as latent TB infection (LTBI) which constitutes 1/3rd of the world's population, ~10% of immunocompetent infected individuals progress towards active disease during their life-time. However, factors that promote this transformation aren't clearly understood. Additionally, IGRA + status cannot differentiate between TB and LTBI. The aim of our study was to identify new biomarkers that will help distinguish between different TB stages.

Methods & Materials: We evaluated the immunogenicity of 22 Mtb antigens, which included PPD, ESAT6/CFP10, Ag85A/B and TB10.4 (immunodominant antigens) together with novel latency (DosR regulon) antigens, resuscitation-promoting factors (rpf), reactivation-associated, starvation-induced and secreted antigens. We enrolled 10 subjects from each of LTBI, active pulmonary (PTB) and extrapulmonary tuberculosis (EPTB) and healthy IGRA- subjects served as controls. Whole blood was stimulated with antigens for 7 days followed by IFNγ ELISA on the supernatants.

Results: Latency antigens (Rv1733, Rv1737 and Rv2029) can be used to discriminate between LTBI and PTB patients due to 2-4 fold difference in their IFNγ responses. EPTB patients showed significantly higher IFNγ responses compared to LTBI and PTB patients to latency antigens (Rv2626 and Rv2628), RpfA (Rv0867) and RpfD (Rv2389), IVE-TB antigens (Rv1806, Rv2034 and Rv3353) and reactivation antigen (Rv1131). Rv0934, Rv0440 and TB10.4 elicited highest IFNγ response in EPTB patients and a pronounced response in LTBI compared to PTB patients; whilst responses to ESAT6/CFP10 and PPD were observed in all TB-infected groups. IGRA healthy controls showed a comparatively weaker response to most of the antigens (<40pg/ml).

Conclusion: IFNγ ELISA is a cheap and useful tool for screening potential antigenicity in subjects with different TB-infection states. Combined use of these novel Mtb antigens has the potential to accurately classify latent versus active TB subjects and may serve as promising biomarkers in the future.
Repurposing of old drugs: Identification of novel sila analogues of rimonabant as potent antitubercular agents


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Background: Tuberculosis is an infectious disease caused by various strains of mycobacteria, the most common one being Mycobacterium tuberculosis (Mtb). Almost one-third of the total world population is infected by Mtb and it is the second leading cause of death due to an infectious agent. Medications are known for treating TB, but they take long time and development of resistance to known antibiotics is another serious problem. In view of all these challenges, there is a need to develop new drug candidates with novel mechanisms for treating tuberculosis. The pre-clinical candidate BM212 was reported to be active against Mtb with an MIC of 1.5 mg/mL. BM212 belongs to the MmpL3 class of inhibitors which stops the transport of mycolic acids.

Methods & Materials: The structural similarity between an MmpL3 inhibitor BM212 and a cannabinoid receptor modulator rimonabant prompted us to investigate the anti-tubercular activity of rimonabant and its analogues. Accordingly several compounds were synthesized and their activity was evaluated against Mtb.

Results: Further optimization, particularly through incorporation of silicon into the scaffold resulted in new compounds with significant improvement in anti-tubercular activity against Mycobacterium tuberculosis (H37Rv).

Repurposing of rimonabant

Conclusion: A sila analogue turned out to be the most potent antimycobacterial compound (MIC, 31 ng/mL) from this series with an excellent selectivity index. Optimization of the series to improve its ADME properties is currently in progress.
Orbital tuberculosis: Clinical and microbiology profile

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Background: *Mycobacterium tuberculosis* infection of the eye is common in India, but the orbital infection is extremely rare. Orbital tuberculosis occurs as a result of hematogenous spread or direct extension from the neighboring structures. In this study we have reviewed the clinical and microbiology features of orbital tuberculosis.

Methods & Materials: Medical and Microbiology records of patients with orbital tuberculosis positive for *M. tuberculosis* in culture between June 2010 and May 2015 reviewed. The study data included demographic details of the patients, clinical presentation, interventions and reports of other investigations performed. Incision biopsy/Pus aspirate/FNAC specimen obtained from patients were subjected to direct microscopy by Ziehl Neelsen staining, inoculated on to Lowenstein Jensen (LJ) medium and histopathology.

Results: A total of 6 patients with orbital tuberculosis were identified during the study period. Four of six patients were females. The age ranges of the patients from 7 years to 29 years. Four patients were less than 15 years, two patients were within 15 to 30 years. All the patients were immunocompetent. Two patients presented with lacrimal gland mass with proptosis, one patient with chronic orbital cellulitis, one patient with orbital cellulitis and tuberculosis osteomyelitis, one with upper lid mass with choroidal granuloma and one patient with upper lid mass. Mantoux test was performed in 4/6 patients and positive in all the four patients. Chest X was performed in 5/6 patients and no abnormality was noted in all these patients. ESR was elevated in 3/5 patients. Histopathology diagnosis was available for 5 patients and 4/5 patients showed granulomatous inflammation with caseous necrosis, one with tuberculosis osteomyelitis. Direct smear was positive for acid fast bacilli and culture grew *Mycobacterium tuberculosis* on LJ medium in all the patients. All the patients were started on anti tuberculosis treatment.

Conclusion: Orbital tuberculosis should be considered as a differential diagnosis in immunocompetent patients with orbital swelling and abscess in TB endemic countries. In majority of patients orbital tuberculosis occurs without systemic involvement.
The urgency of effective antitubercular drug development – new promising structures derived from natural terpenoids

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Background: Despite the availability of highly efficacious treatment for decades, tuberculosis (TB) remains a major global health problem. The widespread transmission of resistant variants of Mycobacterium tuberculosis, which does not respond to any of the commercial drugs, threatens health security of both developed and developing world. The urgent need of new antimycobacterial agents and development pathways is becoming more and more apparent.

Methods & Materials: More than 200 new diverse structures, including more than 50 new synthetic chiral compounds derived from natural terpenoids (+)-camphor and (-)-fenchone were synthesized. The compounds were evaluated for their in vitro antimycobacterial activity by proportional method against reference strain Mycobacterium tuberculosis H37Rv and multidrug resistant Mycobacterium tuberculosis strain 43.

Results: The quantitative structure–activity relationship (QSAR) revealed several structural requirements: two hydrogen bond donors, two or three rings and no large branched substituents. We describe the design of a set of nine novel camphane-based derivatives following these requirements. Four of them showed activities in the nanomolar range, significantly higher than the activities in the initial set. Many structures showed promising antimycobacterial activity (MIC up to 0.27 µM) – 10 to 20 fold higher than activity of ethambutol in combination with insignificant cytotoxicity (IC₅₀ more than 354 µM toward human embryonal kidney cell line).

Conclusion: When developing new drugs against Mycobacterium tuberculosis, it is important to note that this bacteria has a solid and chemically resistant cell wall. Therefore, anti-TB agents are specific and do not act on other pathogenic bacteria, and vice versa - the huge variety of available antibiotics does not affect the mycobacteria. For all tested compounds there is no correlation between their antimycobacterial activity and activity against other microorganisms. This indicates that the action of all potent derivatives (+)-camphor and (-)-fenchones) are specific to the Mycobacterium tuberculosis. All of them are stable, non-toxic against human cells and show antimycobacterial activity in the nanomolar range being 60 times more active than ethambutol. These results can be considered an important starting point for design of new effective antitubercular drugs.
Genotypes of mycobacterium tuberculosis isolated from blood of active tuberculosis patients

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**Background:** Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. Mycobacteremia is a key event in the pathogenesis of tuberculosis and development of extrapulmonary TB. Genotyping of *M. tuberculosis* isolates is useful for surveying the dynamics of TB infection, identifying new outbreaks, and preventing the disease. We attempted to estimate the prevalence of *M. tuberculosis* bacteremia and its genotypes.

**Methods & Materials:** Suspected cases of tuberculosis were screened for mycobacteremia by culturing 5 ml of peripheral blood sample in the MB/BacT® culture system (bioMérieux, France). Flashed positive bottles were screened for *M. tuberculosis* using *in-house* multiplex PCR and further screened for drug susceptibility. All isolates were subjected to spoligotyping, 24 loci MIRU-VNTR, TbD1+ and RD analysis. Genotyping analysis was performed using SIT-VIT web and MIRU-VNTRplus online tools.

**Results:** Blood culture was performed from 469 patients (306 extrapulmonary, 50 pulmonary and 113 disseminated TB cases). Seventy seven (16.4%) of these blood cultures were positive by MB/BacT®. Of the 77 isolates, 36 (11.7%) were patients had extrapulmonary TB, 34 (30%) disseminated TB, and 7 (14%) pulmonary tuberculosis. Among the culture positives 67 (87%) were identified as *M. tuberculosis* and 10 (13%) as mycobacteria other than tuberculosis using *in-house* multiplex PCR. Spoligotyping identified 65 isolates (97%) belong to 16 previously described Share Types, while the remaining two isolates had unique/un-identified Share Types patterns based upon SIT-VIT web. Among them ST26 (CAS1_DEL) was more predominant (49.2%) followed by ST25 (CAS1_DEL) (9.2%), ST1 (Beijing) (9.2%) and ST19 (EAI2_MANILLA) (7.6%). Largest number of clustering of isolates was detected in Beijing (100%), CAS (97.4%), and EAI (81.8%) by spoligotyping. TbD1 analysis showed that majority of the isolates (83.5%) were modern lineages (TbD1−) and 11 (16.4%) isolates belong to ancient lineage (TbD1+). All the ancient lineage isolates belong to EAI lineage.

**Conclusion:** CAS lineage was predominantly found among mycobacteremia patients. This pattern is not different from the predominance of mycobacterial isolates genotyped from pulmonary TB cases.
Diagnostic performance of RT-qPCR method by targeting 85B mRNA in the laboratory diagnosis of Mycobacterium tuberculosis infection: A preliminary study in Turkish patients

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Background: The problems with any PCR method using DNA sequences as targets for amplification include false-positive results, the inability to detect a difference between viable and nonviable organisms and the inability to determine drug susceptibility. Instead of DNA detecting assays, using RT-qPCR to assess the expression of the 85B mRNA gene of M. tuberculosis indicates the presence of a viable organism. In this study, we used the mRNA coding for 85B antigen complex which is present in all mycobacteria. We aimed to assess the diagnostic performance of RT-qPCR method by comparing with the real-time PCR Cobas TaqMan MTB kit.

Methods & Materials: A total of 54 cases including 32 (17 males, 15 females) patients with confirmed tuberculosis and 22 (13 males, 9 females) individuals without tuberculosis were included as patient and control groups, respectively. The mean ages of 30 males and 24 females were 37.06 ± 8.88 and 36.75 ± 8.96, respectively. Decontamination with N-acetyl-L-cysteine and sodium hydroxide (NALC–NaOH) at 2% and Mycobacterial Growth Indicator Tube (MGIT) 960 system was used for the laboratory diagnosis of sputum samples. Decontaminated samples were used for the manual extraction of M. tuberculosis DNA and 85B mRNA. Extracted DNA was used for Cobas TaqMan MTB DNA qPCR kit (Roche Diagnostics) in a cobas TaqMan 48 analyzer. For RT-qPCR method, total RNA was extracted from the sputum specimen using standard TRIzol protocol (Invitrogen) and 85B mRNA analyses were performed on LightCycler 480 II system.

Results: A significant difference was detected between patients and control groups by RT-qPCR method (p<0.001) and Cobas TaqMan MTB DNA qPCR kit (p=0.0027). Sensitivity, specificity, positive and negative predictive values and kappa coefficient were determined as 97%, 95%, 97%, 95%, 0.89% for RT-qPCR method and 91%, 86%, 91%, 86%, 0.96% for Cobas TaqMan MTB DNA qPCR kit, respectively.

Conclusion: Although, our preliminary results indicated that the diagnostic performance of mRNA-based RT-qPCR method is slightly better than the commercial DNA-based diagnostic qPCR assay, we may come to a more definite conclusion after the completion of the study.
Head-to-head comparison of two multi-locus sequence typing (MLST) schemes for characterization of Acinetobacter baumannii outbreak and sporadic isolates

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**Background:** Acinetobacter baumannii is a frequent cause of nosocomial infections, in particular ventilator-associated pneumonia, urinary tract, and bloodstream infections. Patients in high-dependency care are mostly affected, and increasing multi-drug resistance worldwide is a cause of growing concern.

**Methods & Materials:** To compare the two Acinetobacter baumannii multi-locus sequence typing (MLST) schemes and to assess their suitability to aid in outbreak analysis we investigated the molecular epidemiology of 99 Acinetobacter baumannii isolates representing outbreak-related and sporadic isolates from 24 hospitals in four different countries (Germany, Poland, Sweden, and Turkey). Pulsed-field gel electrophoresis (PFGE) was used as the reference method to determine the epidemiologic relatedness of isolates and compared to MLST using both the Oxford and Pasteur scheme. Rep-PCR was used to define international clonal lineages (IC).

**Results:** We identified 26 unique outbreak strains and 21 sporadic strains. The majority of outbreaks were associated with carbapenem-resistant A. baumannii harbouring oxacillinase OXA-23-like and corresponding to IC 2. Sequence types (STs) obtained from the Oxford scheme correlate well with PFGE patterns, while the STs of the Pasteur scheme are more in accordance with rep-PCR grouping, but neither one is mirroring completely the results of the comparator. On two occasions the Oxford scheme identified two different STs within a single outbreak where PFGE patterns had only one band difference. The CCs of both MLST schemes were able to define clonal clusters that were concordant with the ICs determined by rep-PCR. IC4 corresponds to the previously described CC15 Pasteur (=CC103 Oxford).

**Conclusion:** It can be concluded that both MLST schemes are valuable tools for population-based studies. In addition, the higher discriminatory power of the Oxford scheme that compares with the resolution obtained with PFGE can often aid in outbreak analysis.
Non-tuberculous mycobacterial empyema in an immunocompetent child
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**Background:** Non-tuberculous mycobacterium (NTM) species are free-living organisms that are ubiquitous in the environment. There are about 100 different species of mycobacteria of which few species cause human disease in immunocompromised individuals. The spectrum of disease caused by NTM range from pulmonary disease including cavity, consolidation, bronchiectasis, lymphadenitis, skin, soft tissue and injection site infections to disseminated disease in immunocompromised individuals.

**Methods & Materials:** We report a 9 years old immunocompetent female patient who presented with *Mycobacterium avium intracellulare* (MAI) empyema. There have been only two reports to our knowledge regarding NTM in immunocompetent children causing empyema both of which were due to mycobacterium chelonae.

**Results:** A 9 years old girl presented with left sided chest pain for 3 months. There was associated intermittent fever and cough for the last two weeks. Chest X-ray (CXR) revealed massive left sided pleural effusion. Pleural tap drained pus. She underwent thoracotomy and decortication. HIV Elisa was negative. TB MGIT culture at the end of 3 weeks grew non-tuberculous slow growing mycobacteria. Line probe assays revealed MAI. A thorough immune workup showed normal serum levels of IgG and IgM and lymphocyte subset assays. Child was started on clarithromycin, isoniazid, rifampicin and ethambutol. A repeat chest X-ray done after two months showed complete resolution of pleural fluid. Treatment is continued and child is on regular follow up.

**Conclusion:** MAI can lead to tuberculous empyema even in immunocompetent children.
Ocular tuberculosis masquerading as retinoblastoma in a young boy
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**Background:** Ocular involvement in childhood tuberculosis is rare. We report a case of intraocular tuberculosis in a young boy who presented with ocular discomfort and blurring of vision and was diagnosed as retinoblastoma preoperatively. Child underwent enucleation and a diagnosis of tuberculosis was made postoperatively.

**Methods & Materials:** In this report we stress the need of consideration of ocular tuberculosis as one of the differentials of retinoblastoma.

**Results:** A six-year-old boy was referred to our TB clinic in June 2015 from cancer hospital for evaluation. This child presented to cancer hospital with complaints of blurring of vision gradually progressing to loss of vision in the right eye for two months. On examination he had leukocoria with minimal perception of light in the right eye. There was significant past history of taking anti TB drugs for multi drug resistant Pott's spine in 2012 for a period of 2 years. Anti TB drugs were stopped in 2014 and child was doing well for one year after stopping medicines. For these new complaints child underwent CT scan of brain and orbits (fig 1) which was suggestive of 5 x 10 mm mass in posterior aspect of right orbit with retinal detachment and mild vitreous haemorrhage which was suspected to be retinoblastoma with normal brain parenchyma. B scan ultrasound of right orbit showed dumbbell shaped choroidal mass lesion, most likely choroidal hemangioma or melanoma. Slit lamp examination revealed yellow white mass lesion in posterior segment behind lens with subretinal seeds suggestive of retinoblastoma. FNAC of the mass was deferred in view of high suspicion of retinoblastoma and risk of spilling/seeding tumour due to procedure. Post operatively histopathology revealed necrotising granulomatous inflammation suggestive of TB with no evidence of malignancy. Child was started on second line anti TB drugs. MRI spine, USG abdomen and chest X-ray did not show tuberculosis.

**Conclusion:** Ocular TB may mimic retinoblastoma and very careful assessment for TB may be required prior to enucleation.
Fluorescent In Situ Hybridization (FISH) for the detection and differentiation of mycobacterium tuberculosis and NTM in sputum and culture

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Background: Early detection and differentiation between Mycobacterium tuberculosis (MTB) and the atypical Mycobacteria play an important role in the treatment and management of patients. We have recently developed ID-FISH Mycobacterium genus specific and MTB complex specific Combo Test Kit (M-genus-TB kit) that detects viable Mycobacteria and differentiates MTB complex from NTM on a single smear using Fluorescent In Situ Hybridization (FISH) technique. The test can be performed on processed sputum samples, liquid cultures, and solid cultures.

Methods & Materials: 200 decoded sputum samples collected from hospitals between January and June 2014, were processed with ReaSLR-CSM sample processing methodology (ReaMetrix, Bangalore, India) and cultured on BBL-MGIT and Lowenstein Jensen (LJ) medium. All slides were fixed with 5% phenol in 70% ethanol. FISH was performed on all processed sputum and positive-culture smears using the M-Genus- TB kit. Fixed smears were treated with a pre-treatment buffer, rinsed, and then hybridized with fluorescently labeled Mycobacterium genus specific (green) and MTB complex specific (orange) probes. After removing excess probes by washing, the smears were read at 1000x magnification using an LED microscope with blue and green filter sets. All cultures were confirmed by sequencing. Ziehl-Neelsen (ZN) staining was done on all slides for comparison.

Results: Of 200 samples, 84 were culture-positive. 83 of the 84 were confirmed as Mycobacteria and 61 of the 83 were confirmed as MTB by sequencing and FISH. 68 of the 83 culture-confirmed sputum samples were positive by Mycobacterium genus specific FISH; and 60 of these were positive by ZN. 54 of the 61 MTB culture-confirmed sputum samples were also positive by MTB complex specific FISH whereas 50 were ZN positive. 12 culture-negative samples were positive by Mycobacterium genus specific FISH. Eight samples positive for Mycobacterium genus specific and MTB complex specific FISH were confirmed positive for NTM by culture. Based on these results, sensitivities for the M. Genus and MTB FISH assay for sputum were 82% and 89%; and specificities were 91% and 95% respectively.

Conclusion: FISH can be a powerful diagnostic tool for the detection and differentiation of TB from NTM infections in resource-constrained settings.

Conflict of interest: NONE declared.
Prevalence and predictors of tuberculosis among adults with newly diagnosed HIV/AIDS

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**Background:** Tuberculosis and HIV co-infection not only presents a challenge in diagnosis and therapy but also constitutes a high burden on healthcare systems. A survey was designed to study the prevalence of and risk factors for tuberculosis among antiretroviral-naive HIV-infected adults.

**Methods & Materials:** A cross-sectional study was conducted among 2866 HIV patients from 10 provinces in China during 2009 to 2010. Clinical and laboratory investigations including chest X-ray, acid fast staining and culture were used to identify tuberculosis cases. Blood samples were collected to determine CD4+ lymphocyte count. A structured questionnaire was used to collect socio-demographic characteristics of study subjects. Factors associated with the presence of tuberculosis were analysed by logistic regression.

**Results:** Among the 2866 patients, 75.3% were male. Median age was 40 years. 29.8% had tuberculosis, 23.0% had pulmonary tuberculosis and 11.9% had extrapulmonary tuberculosis. The prevalences of smear-positive pulmonary tuberculosis and of culture-positive pulmonary tuberculosis were 11.8% and 17.2% respectively. Tuberculosis was more prevalent among men, ethnic minority patients, patients with CD4 count of < 200/mm3, and patients who were < 50 years of age. The prevalence of tuberculosis differed significantly according to province and HIV transmission route. Tuberculosis was more common in patients with fever, cough, night sweats, fatigue, weight loss, loss of appetite, abnormal pulmonary imaging findings, and history of tuberculosis. In multivariate analysis, having been diagnosed in provinces Henan (OR=46.863), Jiangxi (OR=8.103), Shanghai (OR=2.273) and Xinjiang( OR=29.451), male sex (OR=1.333), ethnic minority (OR = 1.620), lower CD4 count (OR=1.382), abnormal pulmonary imaging (OR = 3.539), fever (OR =3.947), cough (OR=2.223), night sweats (OR = 4.461), weight loss (OR = 1.830), and history of tuberculosis (OR=3.712) were associated with increased adjusted odds of tuberculosis among HIV patients.

**Conclusion:** Tuberculosis is highly prevalent among Chinese adults with newly diagnosed HIV/AIDS. Geographical areas, male sex, ethnic minority, lower CD4 count, having abnormal pulmonary imaging findings, history of tuberculosis, and presenting with non-specific symptoms including fever, cough, night sweats or weight loss were found to be the predicting factors for tuberculosis among HIV-infected patients.
Molecular characterization of mycobacterium tuberculosis strains isolated in India


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Background: Genotypic analyses of Mycobacterium tuberculosis are essential in understanding its epidemiology and transmission in a region. Spoligotyping and 24 Loci MIRU-VNTR are widely used PCR based rapid methods for genotyping M. tuberculosis complex (MTB) strains. Only few in-depth analyses of the population structure of MTB is known from India. In this study we executed genotyping of M. tuberculosis complex clinical isolates from different geographical regions of India.

Methods & Materials: A total of 628 M. tuberculosis isolates were collected from 9 different locations of India viz: New Delhi (n=64,10.2%); Agra (n=59,9.4%); Punjab (n=61,9.7%); Mumbai (n=112,17.8%); Nagpur (n=52,8.3%); Hyderabad (n=94,15%); Chennai (n=50,8%); Kolkata (n=75,12%), and Assam (n=61,9.7%). All the isolates were subjected to Spoligotyping and 24 loci MIRU-VNTR and their patterns were analysed using SIT_VIT WEB2 and MIRU-VNTR plus respectively. Clustering and diversity analysis (Hunter-Gaston Diversity Index) were also performed.

Results: Spoligotyping analysis detected 102 distinct spoligo-patterns. A total of 536(85.3%) isolates could be grouped into 85 SITs which matched the pre-existing database. For 34(5.4%) ungrouped isolates 17 new SITs were created, and for the remaining 58(9.2%) isolates no SIT number could be ascertained and these were considered as ‘orphan’. All the 58 orphan strains were analysed using 24 loci MIRU-VNTR and appropriate genotypes were identified. Overall using both techniques, CAS family was predominant, comprising of 253(40.3%) isolates, followed by EAI in 172(27.4%), Beijing in 110(17.5%), Manu in 41(6.5%), T in 31(4.9%), H in 11(1.8%), X in 4 (0.6%), Africanum in 4(0.6%) and Ural in one. Using the HuntereGaston diversity index (HGI), the allelic diversity of the loci MIRU10, MIRU 16, MIRU 23, MIRU27, MIRU31, Mtub04, Mtub21, Mtub30, QUB11b, QUB26 and QUB4156 were highly discriminant.

Conclusion: CAS was most predominant in Northern and western parts of India viz: Agra, Delhi, Punjab, Mumbai and Nagpur while EAI was predominant in Chennai, Hyderabad. Highest prevalence of Beijing was found in Assam and Kolkata. Our data clearly demarks the prevalence of specific M. tuberculosis genotypes among different geographical regions.

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Interleukin-6: a potential biomarker of the success of tuberculosis treatment

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**Background:** The present measure of the success of TB treatment is fraught with problems. A cytokine biomarker(s), a qualitative and quantitative reflection of the treatment success, is thus warranted. Because TB treatment is expected to affect macrophage and mycobacteria interactions and, consequently cytokine(s) elaboration, a biomarker(s) seems crucial to assess its success.

**Methods & Materials:** We studied the effect of anti-TB drugs isoniazid (INH), rifampicin (RIF), streptomycin (STP) and ethambutol (EMB) on IL-1β, IL-6, IL-10, IL-12 p40 and IL-12 p70 elaboration by mouse peritoneal macrophages (PMs) infected with *M. tuberculosis* H37Rv for 6 h, 24 h, 4 days and 7 days, *in vitro*, by using a multiplex suspension cytokine array system.

**Results:** INH, at 6 h, at 0.4 µg/ml (MIC: 0.2 µg/ml) and not at 0.1 and 0.2 µg/ml, and at 24 h and on D 4 and D 7, at all the tested concentrations, significantly (p < 0.001) suppressed IL-6 elaboration. RIF, at 6 h, lacked any effect on IL-6 elaboration at 0.4 µg/ml (MIC: 0.8 µg/ml), whereas at 0.8 and 1.6 µg/ml, and at all the tested concentrations at 24 h and on D 4 and D 7, significantly (p < 0.001) inhibited IL-6 elaboration. STP, at all the time-points, at 2.5 µg/ml (MIC: 5 µg/ml), lacked any effect on IL-6 elaboration; however, at 5 and 10 µg/ml it caused significant (p < 0.001) inhibition. Surprisingly, EMB at 4, 8 and 16 µg/ml (MIC: 8 µg/ml) suppressed IL-6 elaboration at all the time-points studied. *M. tuberculosis*-infected untreated controls, in contrast to the uninfected ones, irrespective of the time-points, elaborated significantly (p < 0.001) high concentrations of IL-6 (infected controls, 365±30.41–777.5±74.45 pg/ml; uninfected controls, 15.25±1.77–21±1.41 pg/ml) only. Curiously, all the other cytokines (IL-1β, IL-10, IL-12 p40 and IL-12 p70) were unaffected by the drug treatments.

**Conclusion:** *M. tuberculosis*-infected PMs, *in vitro*, elaborated IL-6 as the only major cytokine, which was significantly inhibited by anti-TB drugs. Therefore, IL-6 can be developed as a potential biomarker or biosignature to assess the success of the treatment of TB.
Diagnosis of human tuberculosis: identification of new biomarker(s) and biosignature(s)

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Background: Globally, human tuberculosis (TB), continues to be one of the deadliest public health problem, despite some recent ground success. According to Global TB Report 2014 World over, TB is showing a gradual declining trend, and during the period 2000 to 2013, an estimated 37 million lives were saved largely due to improvements in its diagnosis and treatment. Nevertheless, because deaths due to TB are considered preventable, it is only necessary that work must continue to further improve on both these fronts. TB is a chronic inflammatory disease caused by the facultative intra-macrophage pathogen Mycobacterium tuberculosis, and their resultant interaction is known to elaborate a wide array of cytokines (stand-alone as biomarkers, and as a group, as biosignatures), which, in turn, are believed to be the reflection of the diseases progression and its status at a particular point in time.

Methods & Materials: We, thus studied the serum levels of interleukin-2 (IL-2), IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), GM-CSF and interferon-γ (IFN-γ) in TB patients, at Day 0, after one month, and after six month, by using a multiplex (Bio-plex) suspension cytokine array. The standard curves for all the cytokines were generated as per the protocol of the supplier.

Results: Results showed that after six month, only IL-2, IL-4, IL-6, IL-8, TNF-α, GM-CSF and IFN-γ showed a major increase; IL-10 showed the least increase only in a few patients. On the other hand, IL-6, IL-8 and GM-CSF showed a maximum increase (IL-6, 140-fold; IL-8, 180-fold; GM-CSF, 140-fold). The remaining cytokines showed relatively lesser increase (IL-4, 1.1-fold; IL-10, 5-fold; IFN-γ, 80-fold and TNF-α, 65-fold).

Conclusion: We conclude that as stand-alone IL-6, IL-8 and GM-CSF may function as potential biomarkers or together, as a group, may be considered as a biosignature for the diagnosis of TB.
Biological evaluation of a novel nitroimidazo-oxazole derivative, IIIM-MCD-019 against Mycobacterium tuberculosis and its in vivo efficacy

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Background: One of the nitroimidazo-oxazole derivatives, IIIM-MCD-019 discovered in-house was assessed for detailed biological activities against various strains of Mycobacterium tuberculosis. Further, *in-vitro* studies such as synergistic activity, intracellular MIC, time kill kinetics, cell cytotoxicity, microsomal stability and pharmacokinetics were performed. *In-vivo* efficacy of the compound was tested alone and in combination.

Methods & Materials: MIC was determined against H37Rv, monoresistant as well multidrug resistant (MDR) isolates and streptomycin starved *M. tuberculosis* (ss18b). Synergistic studies were performed with rifampicin, isoniazid and ethambutol using microdilution checkerboard assay. Time kill of the compound was performed at MIC to 8X MIC against *M. tuberculosis* H37Rv. Intracellular MIC was performed in macrophage J774 cell lines using RPMI-1640 media. Cell cytotoxicity of the compound was assessed on HepG-2 cell lines using glucose and galactose containing media to identify mitochondrial cytotoxicity. Microsomal stability of the compound was performed using rat liver microsomes. PK parameters were assessed in mice by standard protocols and *In-vivo* efficacy was assessed in intranasal model using Balb/c mice for 4 weeks.

Results: IIIM-MCD-019 exhibited an MIC ranged between 0.12-0.25 µg/ml for all strains except for NRP which is streptomycin starved. When combined with rifampicin, isoniazid and ethambutol individually, it showed synergistic effect with rifampicin and isoniazid and additive effect with ethambutol and it has intracellular MIC of 1µg/ml. Time kill studies shows that killing rate of this compound is comparable to the best in class drug candidate i.e. delamanid (OPC-67683). The compound did not exhibit cytotoxicity either in glucose or in galactose and found to be ≥ 99% stable in rat liver microsomes. Pharmacokinetic profile in terms of C max & AUC0-t also shows 1.5 times increase (C max of 0.54 µg/ml and of 7.42 µg/ml* h) compared delamanid. In *in-vivo* model, the compound showed 1 log reduction in cfu wrt early control and better efficacy when combined with combination of rifampicin and isoniazid.

Conclusion: IIIM-MCD-019 is a novel compound from nitroimidazo-oxazole scaffold and has a potent antiTB properties. The compound has shown better PK profile than the drug candidate, however further optimization of structure is required to achieve better *in-vivo* efficacy.
Isolation and identification of a novel Non-tuberculous Mycobacterium species of canine origin by multiple gene sequencing approach

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**Background:** Frequently isolated in clinical settings and environmental sources, members of the Mycobacterium avium-intracellulare complex (MAIC) comprising genetically distinct species and subtypes holds significant agents responsible for opportunistic infections. The work is focused on molecular identification of a novel Mycobacterium species within the MAIC isolated from an aged dog suffering from pulmonary infection in a rural area of Meghalaya, India.

**Methods & Materials:** The dog’s nasal swabs were collected and after decontamination inoculated into Lowenstein Jensen Media. Since phenotypic characteristics were inconclusive and time consuming, molecular analysis was decisively adopted for the study. Genus confirmation was done on the basis of hsp65 gene amplification which was then subjected to PRA (PCR-Restriction Enzyme Pattern Analysis) using enzymes BstEI and HaeIII. To further speciate the isolate and determine its phylogenetic status, two important additional housekeeping genes rpoB, and 16S rRNA gene were included.

**Results:** At the end of three months a smooth, single, non-pigmented colony was observed showing strong acid fast bacilli and amplified the genus-specific hsp65 gene. PRA-hsp65 profiles could only infer the isolate to be a Non-tuberculous Mycobacteria (NTM). The dog was instantly prescribed anti-tuberculosis drug and is currently undergoing therapy. BLAST analysis in NCBI exhibited no homology in the three genes with comparatively low similarity cut-offs. The 16S rRNA gene depicted 99% closeness to the reference strain of *M. yongonense*, rpoB gene showed 97% similarity to *M. Indicus pranii*, while non-MAIC member *M. genevense* was the closest hit for hsp65 with 97% similarity. All the three genes were individually subjected to phylogenetic analysis in MEGA6 taking together the reference ATCC sequences of MAIC and the closest BLAST hits which also presented similar ambiguous output with no conclusion on the isolate’s species. Concatenation of the three sequences ultimately presented higher lucid discrimination and determined the isolate (HNP-1) as a novel entity within the MAIC (Figure 1).

**Conclusion:** Phylogenetic analysis indicate the isolate as a distinct new member within MAIC with a unique clinical manifestation in the present dog’s case by way of being less lethal as compared to other reported cases.
Patients' satisfaction with TB DOTS services in PHC facilities in Katsina State, Nigeria
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Background: In Nigeria, Katsina, tuberculosis (TB) remains a major issue despite the availability of a TB control program and free anti-TB drugs. Assessment of client perception in Tuberculosis programs could contribute to understanding the gaps in health care delivery service and also the specific needs of the patients. This is very important in identifying the barriers to and the facilitators of successful TB control programs. This study demonstrates the perception of patients utilizing DOTS services in primary health care facilities in Katsina state and how it relates to their level of satisfaction.

Methods & Materials: This study was conducted on patients with pulmonary tuberculosis receiving DOTS services in PHC centers in Katsina state. A cross sectional descriptive study was carried out in January 2014 with a sample of 225 patients obtained by a multi-stage sampling process. A structured, interviewer-administered questionnaire was used to assess satisfaction of patients and data was analyzed using SPSS Version 21.

Results: Most of the respondents were males (70.2%), married (66.7%) and Muslim (92.4%) with no formal education (49.3%) and farming constituting their major occupation. Most of the patients were satisfied with different components of the TB control services. Majority of respondents (93%) were satisfied with TB DOTS services. There was statistically significant association between education and satisfaction with TB services (p=0.006) and also between satisfaction and religion (p=<0.0001). No statistically significant association was found between respondent's gender, occupation and their satisfaction with TB services.

Conclusion: Although DOTS in Katsina state services is provided in a resource poor setting, patient's perception of DOTS services was good as they were satisfied with all the components of DOTS services. Future Healthcare interventions activities should hinge on these findings so as to improve service delivery and yield more comprehensive results. In-depth understanding of other factors contributing to satisfaction is also crucial for public health authorities to improve existing healthcare systems and, in turn, benefit the population seeking care.
Knowledge of health care workers on TB and DOTS strategy in PHC facilities in Katsina State, Nigeria

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**Background:** Tuberculosis kills millions of people around the world, and also it threatens the livelihoods of families and communities worldwide with developing countries the hardest hit where 95% of all new TB cases and 99% of deaths occur. A critical strategy for successful TB control is the prompt, appropriate and complete treatment of all patients diagnosed with active disease. This cannot be achieved if health care workers’ knowledge of the disease is deficient or national treatment protocols are not followed. This study explored the knowledge of health care workers on TB and DOTS strategy in Primary Health Care centers in Kastina state.

**Methods & Materials:** A cross sectional descriptive design was employed with a sample of 305 respondents using the multistage sampling method. A structured, self-administered questionnaire with questions mostly adapted from National TB and leprosy control program workers manual was used to explore knowledge of health workers and the data was analyzed using SPSS Version 19. Ethical approval was obtained from the Ethical review board of the Kastina state Ministry of Health while written informed consent was obtained from all respondents.

**Results:** Less than half (43%) of the respondents had adequate knowledge on TB and DOTS services. Only 13% and 15% of the respondents knew the correct meaning of DOTS and DOT respectively. Only 24.3% of the health care workers had received training on TB control. There was statistically significant association between designation of health workers and knowledge of TB and DOTS Strategy (p=<0.001). There was no statistically significant association between knowledge and training, number of years spent in health facility.

**Conclusion:** The study reflects that knowledge of TB and DOTS strategy was poor. The state control program in collaboration with TB control supporting partners in the country, should improve training of all the health workers on TB control services.
Latent tuberculosis infection among close contacts of non-residential pulmonary tuberculosis patients in Shanghai, China
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Background: Under the fast urbanization, Shanghai is hosting more and more domestic rural-to-urban migrants who do not have a certified local residence. Tuberculosis is more prevalent in rural population in China. In 2014, non-residential population has accounted for 42.9% of new pulmonary tuberculosis (PTB) patients in Shanghai. Close contacts of non-residential patients are at high risk of latent tuberculosis infection (LTBI). This study aimed to understand the prevalence of LTBI in close contacts of non-residential PTB patients, and to identify the risk factors associated with LTBI in Shanghai.

Methods & Materials: A cross-sectional study was conducted among close contacts of non-residential PTB patients diagnosed in 2013-2014 in 4 districts of Shanghai. T-SPOT.\text{TB} was applied to detect the LTBI among contacts, together with a questionnaire for collecting information on demographics, socio-economic status, history of Bacille Calmette-Gue\’rin (BCG) vaccination, symptoms of TB and details of contacting. The status of LTBI was defined as T-SPOT.\text{TB} positive plus no TB symptoms and a normal lung image by chest X-ray.

Results: In this study, 460 close contacts were self-reported by 226 registered PTB patients. Of these contacts, 43.0% were male and 58.0% were BCG vaccinated. Overall, 83 contacts had positive T-SPOT.\text{TB} results without TB symptoms, which presented an 18.0% (95%CI: 14.5%–21.6%) prevalence of LTBI. The prevalence of LTBI increased with age ($\chi^2_{\text{liner trend}}=3.910$, $p=0.048$), and exposure duration to PTB patients ($\chi^2_{\text{liner trend}}=6.401$, $p=0.011$). Stratified analysis by age (0-19, 20-39, 40-59, and $\geq$60 years) indicated that the association between LTBI prevalence and exposure duration was statistically significant at the age of 20-39 years ($\chi^2_{\text{liner trend}}=4.947$, $p=0.026$). Multivariate analysis showed that household contact significantly increased the risk of LTBI (aOR=9.030, 95%CI: 2.568-31.756); and contacts of PTB patients having cough (aOR=2.541, 95%CI: 1.258-5.133) and cavities in lung (aOR=1.698, 95%CI: 1.008-2.860) were more likely to be LTBI than those otherwise.

Conclusion: Close contacts of non-residential PTB patients had a relatively high LTBI prevalence. Intervention for infection control among PTB close contacts should be concerned in the policy development for ending TB in 2035 in Shanghai.
The serum Th1 and Th2 cytokines levels in active tuberculosis patients before and after 2 month anti-TB treatment
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Background: Pulmonary tuberculosis (TB) remains a major public health burden in China. It is generally thought that while B cell- and antibody-mediated immunity plays an important role in host defense against extracellular pathogens, given the role of cell-mediated immunity (CMI) in providing protection against TB, this study aims to investigate the levels, impact factors and variations of T helper 1 (Th1) (IFN-γ, interleukin (IL)-2), and Th2 (IL-4, IL-10) cytokines in pulmonary TB patients before and the end of 2 month anti-TB treatment.

Methods & Materials: The active TB case-cohort was established in five districts designated TB diagnosis hospitals in Shanghai. All registered active TB patients diagnosed during 2013 to 2014 were investigated using a structured questionnaire covering geographic, demographic, social-economic information and disease profile. Enzyme-linked immunosorbent assay (ELISA) was used to assess the level of serum IFN-γ, IL-2, IL-4, and IL-10 before and 2 month later of the treatment.

Results: Overall 309 TB patients were enrolled, among which 72.49% (224) were male, and 78.64% (243) were sputum smear positive (SS+). The average age was 51.43, varied from 17 to 91. The sputum smear negative conversion rate at the end of 2 month anti-TB treatment was 75.72%. The mean of serum IFN-γ, IL-2, IL-4, IL-10 levels of participants before anti-TB treatment were 43.30, 14.24, 43.21, and 29.33 pg/ml, respectively. The IFN-γ, IL-2, and IL-10 were significantly decreased after 2 month anti-TB treatment (IFN-γ: 36.41 pg/ml, p=0.003; IL-2: 39.14 pg/ml, p<0.001; IL-10: 12.31 pg/ml, p<0.001). The cytokines rate of IFN-γ/IL4 before and after 2 month anti-TB treatment was 3.56 and 2.16, respectively. There was no significant difference of cytokines rate between before and after 2 month treatment.

Conclusion: Th1 and Th2 cytokines may be involved in the development of pulmonary tuberculosis and impact on the prognosis of disease. Tracking the serum levels of relative cytokines may be helpful to explore the course of pulmonary tuberculosis and evaluate the efficacy of the treatment.
A quick method to determine the best threshold level for universal vaccination when there is an outbreak of Japanese Encephalitis

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**Background:** Japanese Encephalitis (JE) has an increasing trend in many parts of the world, especially the global warming facilitates the perpetuation of the vectors, mosquitoes. Although JE vaccination is a proven useful control strategy, at the same time it has a not low associated complication rate. What is the best time to opt for universal vaccination need a careful balance.

**Methods & Materials:** A risk vs benefit approach is used to determine the best time to opt for universal JE vaccination, using Hong Kong as an example. Two sources of information are used to determine the case loads / endemicity of JE (as a proxy to estimate the potential benefit for vaccination): (1) Seroprevalence in different population subgroups; (2) Surveillance information from the Government Disease Control Centre under the WHO IHR. Two sources of information are used to assess the potential risks for vaccination: (1) Complication rate for JE vaccination based on pharmaceutical companies' data; (2) Acceptability, knowledge and attitude of JE vaccination in the public.

**Results:** The baseline case load of JE in Hong Kong is not high. However, certain subgroups are at high risk (e.g. elderly) as reflected by a higher seroprevalence rate. Universal vaccination may be justified only if the annual incidence is higher than the damage potentially caused by the vaccination itself.

**Conclusion:** There is a need to review the surveillance process since updated data is the crux for an accurate assessment. Even if there is an universal JE vaccination program, the responsible authority (e.g. government) has to address the concern from the public in order to achieve a satisfactory coverage for significant community protection.
Clinical Review of Shanchol™ (a WHO pre-qualified oral cholera vaccine)

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Background: Cholera caused by *Vibrio cholerae* of the O1 or O139 serogroup is an important cause of severe dehydrating diarrhea in adults and children. It continues to be a major global health problem. The WHO estimates that 3-5 million cases and 100,000–130,000 deaths occur per year globally, predominantly in Asia and Africa.

The WHO recommends that in cholera-endemic countries, vaccination should be used as an additional tool to control cholera along with longer term interventions of improving water and sanitation. It recommends pre-emptive vaccination to help prevent potential outbreaks or the spread of current outbreaks.

Shanchol™ is a ready-to-use killed bivalent (O1 and O139) whole-cell oral cholera vaccine administered as 2 doses 14 days apart in individuals aged 1 year and above. There is no need for buffer or water for its administration. It is currently licensed in India and 17 other countries in Asia, Africa and Latin America. It is prequalified for international use by the WHO. The vaccine is the basis for the WHO stockpile which is used to help control cholera epidemics.

Methods & Materials: Clinical analysis of safety, immunogenicity and efficacy studies of Shanchol™

Results: Overall, ~66,000 doses have been administered to subjects during the clinical development of the vaccine. Shanchol™ was observed to provide sustained protective efficacy level of 65% for at least 5 years in a clinical trial conducted in over 69,000 subjects in India. The herd effect conferred by Shanchol™ was evident with minimum vaccine coverage of 38% and was sustained for at least 3 years.

Safety and immunogenicity clinical trials conducted in India, Bangladesh, Ethiopia, Philippines and Haiti showed that the vaccine is safe and provides good immune responses in different endemic populations.

Results from a clinical study showed that flexible dosing schedule of Shanchol™ as 2 doses 14 to 28 days apart is possible. A modeling study observed that single-dose vaccination may prevent more deaths during outbreaks than 2-dose vaccination.

Shanchol™ was also observed to be highly effective in mass vaccination campaigns in Guinea, Haiti, Odisha (India) and Bangladesh.

Conclusion: Shanchol™ vaccine is safe and effective for control of cholera in endemic countries
Vero cell derived novel inactivated Japanese encephalitis vaccine JENVAC
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Background: Japanese Encephalitis (JE) is a mosquito-borne disease caused by JE virus belongs to the family Flaviviridae. In Asia approximately 68,000 clinical cases among 10,000 deaths occurs every year. Although symptomatic JE is rare, the case-fatality rate among those with encephalitis can be as high as 30%. Permanent neurologic or psychiatric sequelae can occur in 30%–50% of those with encephalitis. The risk is highest in children aged 1-15 years, in rural areas and in the monsoon/post monsoon season.
JE vaccine (JENVAC®) development is a collaborative project between Bharat Biotech International Ltd, Hyderabad, India and National Institute of Virology, Pune, India.

Methods & Materials: Virus strain (821564-XY) used in vaccine development which is isolated from the endemic region of Kolar, Karnataka, India. The world’s first fully-integrated, single use bioreactor used for the production of JE vaccine using Vero cell line. Purification is performed using chromatography techniques. Formulation has been carried out with aluminium hydroxide gel.

Results: Non-clinical toxicology studies were conducted in lab animals and the vaccine has no toxic effects. The safety of this vaccine was established in a Phase-I study on healthy adult volunteers and the results further confirmed in phase II/III study. The efficacy of this vaccine was studied in endemic and non-endemic areas. The sero-protection of the RS.JEV (Chinese vaccine, SA 14-14-2) is 77.56% at 28th day and has a decrease of 5% after 6 months, whereas JENVAC® showing sero-protection of 98.67% at 28th day of single dose vaccination and 99.78% after 56 days with a second dose vaccination. The Immunogenicity study has given 91% at 12 months, 67% at 18 months, 61% sero-protection even after 24 months.

Conclusion: The other vaccines available against JE virus are live attenuated which is derived from the primary hamster kidney cell culture whereas vaccine JENVAC® manufactured by Bharat Biotech is the first VERO cell derived inactivated indigenous vaccine which induces high immunity against JE virus in adults and children above one year and JENVAC® is found stable for 2 years at 2-8°C is commercially available. Based on the results it is concluded that JENVAC® gives long-term protection against Japanese encephalitis infection.
Routine immunization data management need assessment survey of selected health facilities and local government areas in Enugu State, June, 2015

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Background: The National Primary Health Care Development Agency (NPHCDA) with the collaboration of partners is developing an enhanced routine immunization (RI) data collection module to function within the National Health Management Information System (NHMIS) on the DHIS2 platform in order to capture and display key RI indicators by dashboard. To commence implementation, a need assessment survey was conducted in 4 LGAs and 16 HFs to identify the capacity of the M &E, cold chain and LGA immunization officers(LIO) for data entry and reporting through DHIS2 and to assess the availability of NHMIS reporting tools and RI-specific data forms at the HFs and LGA level.

Methods & Materials: We purposively selected 4 LGAs (2 good performing and 2 poor forming) based on NHMIS report on data completeness, timeliness in 2014. We purposively selected 4 HFs in each LGA, making 16 HFs. We qualitatively assessed the needs of the LGA and HFs. We administered semi-structured questionnaire and ODK Collect to LIO, CCO, RI focal person and officer –in- charge to collect data on routine immunization data access, use and quality; stock management data recording; infrastructure and system capacity; data review/analysis and feedback/action on data. Descriptive data analysis was done using Microsoft excel 2010.

Results: A total of 12 HFs and 4 LGAs were assessed. Majority (58.3%) of the health facilities reported that compilation of monthly data report is done by HF officer- in-charge. Majority (58.3% and 50%) of the health facilities reported that the staffs were trained on routine immunization data recording and reporting respectively in the past 12 months. Routine immunization micro plan was not seen in all the HFs visited. Majority (75%) of the HFs receives data recording and reporting tools for RI from the LGA primary health care. Only 8.3% of the HFs makes use of the vaccine management tools. Only 50% of the HFs takes data tools to outreach sessions.

Conclusion: The need assessment revealed a lot of gaps in RI data management that needs to be addressed immediately before the commencement of DHIS2 in the state. RI tools need to be promptly distributed to all health facilities.
Development of new generation blood-stage malaria vaccines against Plasmodium falciparum targeting the PfRH5-CyRPA multiprotein adhesion complex

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**Background:** Erythrocyte invasion by *Plasmodium falciparum* parasites is central to malaria pathogenesis. Thus, it is critical to understand this intricate biological process for the development of novel malaria intervention strategies. *P. falciparum* erythrocyte invasion is a complex, multistep process that is mediated by a number of redundant ligand-receptor interactions. Extensive research over three decades has aimed at identifying an essential parasite ligand that could be targeted across multiple parasite strains. In this regard, PfRH5 was identified as a critical ligand that binds with the erythrocyte receptor, Basigin and elicits potent cross-strain neutralizing antibodies. We have for the first time expressed full-length recombinant PfRH5 in *E. coli* with a structural integrity that mimics the native parasite protein. Our recombinant PfRH5 protein is functional, binds with Basigin and elicits potent cross-strain transcending parasite neutralising antibodies that substantiates its claim as a leading vaccine candidate (IAI, 2014). In addition, PfRH5 lacks a transmembrane domain or GPI anchor and thus a major question has been that how PfRH5 is anchored on the merozoite surface during invasion such that it can engage with its receptor Basigin and mediate attachment. We have successfully solved this conundrum and identified an essential, novel, conserved GPI-anchored parasite molecule, CyRPA that tethers PfRH5 on the parasite surface as a multiprotein complex (PNAS 2015).

**Methods & Materials:** We have produced different fragments of PfRH5/CyRPA recombinant proteins, functionally characterized their binding properties and measured the parasite neutralizing ability of their antibodies.

**Results:** We demonstrate that PfRH5/CyRPA monoclonal and polyclonal antibodies potently block erythrocyte invasion synergistically in a strain-transcending manner by abrogating formation of the PfRH5/CyRPA essential complex. We have thus identified a new conserved mechanism overcoming the previous challenges of blood-stage vaccines (antigen diversity, redundancy, heterologous strain cross-reactivity) paving the way forward to develop new generation blood stage *P. falciparum* vaccines based on PfRH5 and CyRPA.

**Conclusion:** Our previous discovery had elucidated the formation of a novel multi-protein PfRH5/CyRPA complex essential for erythrocyte invasion, which we have validated *in vitro* as a new potent target for the development of novel blood-stage vaccines that could elicit protection alone or in combination with the advanced pre-erythrocytic RTS.S sub-unit vaccine.
Development of Safe, Effective and Immunogenic Vaccine Candidate for Diarrheagenic Escherichia coli Main Pathotypes in Mouse Model

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Background: Enteric and diarrheal diseases are important causes of childhood death in the developing world. These diseases are responsible for more than 750 thousand deaths in children under 5 years old, ranking second cause of death, after lower respiratory diseases, in this age group. Among the major causative agents of diarrhea is Escherichia coli (E. coli). There are several vaccine trials for diarrheagenic E. coli. However, diarrheagenic E. coli has many categories and pathotypes and vaccines are directed for one or two of the five main pathotypes-causing diarrhea. Currently, there are no combined vaccines available in the market for all five diarrheagenic E. coli categories and pathotypes. Therefore, we aimed to develop a low-cost vaccine candidate combining the five main diarrheagenic E. coli to offer wide-spectrum protection. We formulated a formalin-killed whole-cell mixture of enteroaggregative, enteropathogenic, enteroinvasive, enterohemorrhagic, and enterotoxigenic E. coli strains as a combined vaccine candidate

Methods & Materials: We immunized Balb/C mice subcutaneously with 10⁹ CFU of combined vaccine candidate to evaluate survival rate, and we immunized mice groups with combined vaccine candidate and monitored biomarkers levels over six weeks as well as measured responses post challenge with relevant live E. coli

Results: We found a significant increase in survival percent post challenged compared to unimmunized controls (p < 0.0001, 100% survival). We found also significant increase in specific systemic antibodies (IgG), interferon gamma (IFNg) and interleukin 6 (IL-6) levels elicited by combined vaccine candidate especially in the first two weeks after mice immunization compared to controls (p<0.05). We found also that CTB-adjuvanted combined vaccine candidate showed higher IgG and IFNg levels than alum (p<0.05).

Conclusion: Overall, our combined vaccine candidate offered protection from the five main diarrheagenic E. coli pathotypes in a single vaccine using mouse model. To the best of our knowledge, this is the first combined vaccine against the five main diarrheagenic E. coli pathotypes that is cost-effective with promise for further testing in humans.
Vaccines for emerging infections: Chikungunya vaccine

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Background: Chikungunya virus (CHIKV) infection is a febrile illness that is rarely fatal, but causes incapacitating arthralgia that persists in convalescence. Unusual clinical presentations with high morbidity are reported in the recent epidemics. The virus genome is under selective pressure to adapt to arthropod and human hosts to sustain transmission. Vaccination is the most feasible and cost-effective option to block virus transmission during epidemics. However, in the absence of data from deployment of CHIKV vaccine, and lack of reference reagents, the challenge was to qualify a field isolate as vaccine strain, and to define a correlate of protective immunity for clinical development.

Methods & Materials: Phylogenetic reconstruction of CHIKV sequences was performed in MEGA5. Selection was inferred by Codon-based Maximum Likelihood estimates in HyPhy. Genome sequencing and adventitious agents testing were performed by Next Generation Sequencing (NGS). Neutralizing antibodies were estimated by 50% Plaque Reduction Neutralization Test (PRNT₅₀) in CHIKV convalescent sera, vaccine antisera and in cross neutralization studies. Antibody isotypes and avidity were estimated by ELISA. GMP vaccine production was established, and pre-clinical toxicity studies were outsourced to a GLP laboratory.

Results: The study identified a novel E1-K211E mutation under significant positive selection in 2009-2010 isolates which formed a distinct clade within the ECSA (East, Central, and South African) genotype. Vaccine antisera and CHIKV convalescent sera from 2006-2013, cross neutralized Asian and ECSA heterotypic variants from 1963 to 2010. Vaccine virus banks were certified free of adventitious agents by sensitive NGS technology. High immunogenicity was established by iterative inactivation methods and animal testing. The candidate vaccine elicited neutralizing antibodies in animals comparable to the levels in convalescent subjects (GMT, 1021 by PRNT₅₀) (95%CI 763, 1366). Spearman-Karber estimates of median effective and protective doses were established in animal challenge studies. Neutralizing IgG3 isotype and antibodies with high avidity were elicited by the vaccine. In GLP pre-clinical toxicity studies, the vaccine elicited no adverse and arthritogenic effect in animals.

Conclusion: The indigenously developed Chikungunya vaccine using a field isolate of the virus, provides a scientific basis for developing safe, immunogenic and cost-effective vaccine for emerging infections, and to address an unmet healthcare need in developing countries.
An Ebola vaccine candidate based on controlled expression of antigen through the recombinant adenovirus system

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Background: The 2014 outbreak of Ebola haemorrhagic fever (EHF) has claimed > 11,000 lives. No vaccines or antivirals are available for EHF, but are currently the subject of intense investigations. Several vaccines targeting the Ebola virus glycoprotein (GP) are currently under different phases of clinical testing, where GP is delivered through chimpanzee adenovirus (Ad) 3 (GlaxoSmithKline), vesicular stomatitis virus (Merck), human Ad26 and35 (Johnson & Johnson) or human Ad5 (Beijing Institute of Biotechnology and Tianjin CanSino Biotechnology). Among these, adenoviruses are well studied and are considered to be potent inducers of cellular and humoral immunity.

Methods & Materials: A probable drawback of constitutive GP expression as followed by all the Ad vaccine platforms mentioned above is the potential toxicity of GP to the cell substrate, leading to production of recombinant Ad clones with low levels of GP expression. We developed a vaccine candidate based on human Ad5 with controlled expression of GP during the production of the vector. The cell substrate (293 IQ cells; MicrobixBiosystems Inc., Canada) constitutively expresses lac-repressor which suppress the CMV promoter driven expression of GP through a lac-operator. In the absence of lac-repressor (e.g., in Vero E6 and 293 cells), the Ad vectors expressed GP without any control. On addition of IPTG to the 293 IQ cells, the inhibition of the Ad-based GP expression is reversed.

Two clones (AdGP1 and AdGP2) and one control Ad virus were tested in Balb/C mice for immunogenicity. The mice were divided into 8 groups of 3 animals each. Four groups received three doses of PBS or control Ad virus or AdGP1 or AdGP2 through intra-nasal, followed by sub-cutaneous and intra-nasal route while the remaining 4 groups were immunized through sub-cutaneous followed by intra-nasal and intra-nasal route.

Results: The ELISA results indicate that AdGP1 and AdGP2 are immunogenic but there was no significant difference in the immunogenicity in both regimens of immunization. On the other hand, even after three immunizations neutralizing antibody titers to Ad virus were ≤ 1: 200.

Conclusion: Ad vector platform with controlled protein expression can be used for other potentially toxic antigens. Further testing and characterization of the vaccine candidate is underway.
Cost-effectiveness analysis of dengue vaccination in the Philippines

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Background: From years 2001-2010, the Philippines ranked 4th among the ASEAN in annual average reported dengue episodes with 45,409 cases. The WHO aims to achieve 50% reduction in dengue mortality and 25% reduction in morbidity by 2020 through integrating vector control approaches with vaccine introduction. Dengue has yet to be prevented and 20 years of development has finally yielded a candidate vaccine that has reached Phase III efficacy clinical trials: Sanofi Pasteur’s dengue vaccine, a recombinant, live, attenuated, tetravalent dengue vaccine (TDV).

Methods & Materials: This study aims to assess the cost-effectiveness of different dengue vaccination strategies in the Philippines from both a societal and a public payer’s perspective. Coudeville and Garnett’s (2012) dengue dynamic transmission model was populated using Philippine-specific dengue vector, epidemiology, and cost data from literature and records review which were validated through consultations with dengue experts from the field of family medicine, vaccine research, molecular biology, epidemiology, public health, entomology, and infectious diseases.

Results: Main results show that over a period of 5 years, conducting a school-based vaccination program targeting nine year-olds for routine vaccination decreases dengue cases and DALYs lost due to dengue relative to status quo by 24% and 26%, respectively. Expanding the vaccination to more children by adding age cohorts close to nine years such as 10 to 11, 10 to 13, 10 to 15, 10 to 17, and 10 to 20 translates to more DALYs averted and less dengue disease costs the government must shoulder.

Conclusion: Cost-effectiveness threshold prices following cost-effectiveness definition of less than or equal to 1x GDP per capita for the public payer and societal points of view are found to be 13 USD per dose and 24 USD per dose, respectively. This cost-effectiveness threshold set by the Philippines’ Department of Health is more stringent than the WHO recommended cost-effectiveness thresholds.
A poxvirus-based vaccine reduces virus excretion after MERS coronavirus infection in dromedary camels

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Background: The recently emerged Middle East syndrome coronavirus (MERS-CoV) can cause severe and fatal respiratory diseases in humans. Antibodies against MERS-CoV can be found in camels in the Middle East but also outside this region. The high prevalence of circulating MERS-CoV neutralizing antibodies in dromedary camels from different geographic regions may indicate wide circulation of MERS-CoV in camels. The ongoing MERS-CoV outbreak in the Middle East and the lack of treatment options or licensed vaccines is of great concern. Vaccination of camels could potentially prevent the spread of this virus.

Methods & Materials: We vaccinated 4 dromedary camels twice with a 4 week interval with $10^8$ PFU MVA-S via both nostrils and intramuscularly. Four control animals received non-recombinant MVA (n=2) or PBS (n=2). Three weeks post-boost, all animals were tested for presence specific antibody responses by MERS-CoV ELSA and virus neutralization assay (VNT). Next, all camels were challenged with a high dose of MERS-CoV and to study the pathological changes, two animals per groups were euthanized and necropsies were performed at day 4 and 14 pi. The antibody response, and pathology were analyses by MERS-CoV-S or MVA ELISAs, -VNTs, qRT-PCR, virus titration, immunohistochemistry (IHC) and in situ hybridization (ISH).

Results: All vaccinated animal developed detectable serum neutralizing MERS-CoV or MVA specific antibody titers 3 weeks post boost vaccination. No clinical signs were observed in MVA-S vaccinated animals but mild clinical sign and a runny nose were observed in control-vaccinated animals after virus challenge. Interestingly, significant reduction of infectious virus excreted and viral RNA transcripts in the vaccinated animals nose after MERS-CoV challenge was observed as compared with control animals, and these protection correlated with presence of neutralizing antibody to MERS-CoV. In addition, in the nose of MVA-S vaccinated animals at day 4 pi, a few MERS-CoV infected cells were detected by IHC and ISH as compared with control camels. Interestingly, sera from MVA-S vaccinated animals cross neutralized camelpox virus.

Conclusion: Our results demonstrate that vaccination of camels with MVA-S confers protects against MERS-CoV infection. In addition, induction of MVA specific antibody cross neutralize camelpox virus, suggesting that MVA-MERS-S can be used as a dual vaccine in dromedary camels.
Integrated analysis of immunogenicity data from 11 dengue vaccine trials across 14 countries at risk for dengue

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Background: Dengue is a mosquito-borne viral infection with a very rapid global expansion during the last 50 years. This disease has become an important public health problem in Asia and Latin America with over half the world’s population at risk¹. Sanofi Pasteur is developing a recombinant, live, attenuated, tetravalent dengue vaccine (CYD-TDV) for countries at-risk of dengue². The results from CYD-TDV trials are useful to observe the trends in immunogenicity (GMT) titres across various countries.

OBJECTIVES: To assess immunogenicity titres after 3 doses of CYD dengue vaccine in children, adolescents and adults up to 60 years by revisiting pre- and post-vaccination GMTs from Sanofi Pasteur CYD-TDV trials.

Methods & Materials: Dengue neutralizing antibody (Ab) levels were assessed by a plaque neutralization test with a 50% endpoint (PRNT50) for each serotype. In total, 25 clinical studies from Phase I to Phase III have been included in the clinical development plan. Of these 25 clinical studies, the integrated immunogenicity analysis presented here is based on results from 11 trials conducted in 8 Asian countries (Philippines, Indonesia, Malaysia, Vietnam, Thailand, Singapore, Australia, and India) and 6 Latin American countries (Brazil, Colombia, Honduras, Mexico, Peru, and Puerto Rico).

Results: Immune titres increased after 3 doses from baseline, and higher GMTs were observed with increasing age and endemicity in all countries considered at-risk of dengue. Further exploration in older adults in Australia³ vaccinated with 3 doses of CYD-TDV, revealed that both the 18-60 age group (N=655) and the 46-60 age group (N=241) had similar GMTs which were higher than baseline.

Conclusion: Integrated analysis from CYD-TDV trials in children, adolescents and adults up to 60 years of age showed a consistent finding of higher GMTs in the vaccinated arm versus control arm. Subjects who received 3 doses of CYD-TDV elicited a balanced immune response against all four serotypes.


Acknowledgements: The Authors are employees of Sanofi Pasteur; CYD-TDV trials are funded by Sanofi Pasteur. The vaccine was not licensed, at the time of this abstract submission.
Preliminary immuninformatics research for prediction the most immunogenic linear and conformational B-cell epitopes of 14-3-3 antigen in echinococcus granulosus

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**Background:** Cystic Echinococcosis (CE) is one of the most important zoonosis parasite diseases which caused by the larval stage of Echinococcus granulosus (Eg). The Eg14-3-3 protein is a vaccine candidate antigen which exists in different development stages of E. granulosus. The basement of vaccine design strategies is identification the most efficacious epitopes of the antigen. This study presents linear and conformational B cell epitopes of the Eg14-3-3 antigen via computational tools.

**Methods & Materials:** The protoscoleces (PSC) of E. granulosus was aspirated from infected lungs and livers of slaughtered sheep (Tabriz, Iran) and then DNA samples were extracted. The polymerase chain reaction (PCR) was performed using specific primers (forward: ATGTCTTCTCTCAGTAAGCGA and reverse: ATCGGCTTTCTCAGTAAGCGA) and basing on the sequence in GenBank (Access No. AY942149). After sequencing the PCR products, our regional Eg14-3-3 sequence was utilized (the sequence of our local Eg14-3-3 shall be published soon). The linear B-cell epitopes were predicted by Bepipred Linear Epitope Prediction algorithm with threshold 0.35. The conformational B-cell epitopes were predicted using a sequence-based server named CBTOPE which uses the support vector machine (SVM) threshold -0.3, and also the three dimensional (3D) properties of the antigen such as, Relative Solvent Accessibility, Number of Transmembrane Domains and protein tertiary structure prediction. The structural details of Eg14-3-3 which are usable in the epitope-based vaccine design evaluated via SCRATCH Protein Predictor.

**Results:** The Best linear B-cell epitopes were selected based on their length (<9 amino acids) and score (highest), so that the high scales consist of ATEVAEGDMQTT, DTLPEESYK, EQKHDGDAK and TGDERKQASDN. Based on CBTOPE algorithm five high score sequence were found as followed; YVAEFCTGDERKQASDN, ELARKAFDDAELDLPEESYKDA, SDAGDTPAESPPKAD, YMACKLCEQCEYDVMVKA and LSVAYKNVVGAR, so that the bold and underlined amino acids are conformational predicted epitopes. Therefore using the 3D structural parameters the most prominent conformational epitopes were YVAEFCTGDERKQASDN and ELARKAFDDAELDLPEESYKDA.

**Conclusion:** Generally, in current study the many different aspects of Eg14-3-3 B-cell epitopes were analyzed so that achieved data will give crucial details of Eg14-3-3 antigen for designing the new generation of epitope-based vaccines against Echinococcus granulosus.
Nasopharyngeal carriage of streptococcus pneumoniae in children under 5 years of age before introduction of pneumococcal vaccine (PCV 10) in urban and rural Sindh

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Background: Pneumococcal Vaccine -10 (PCV 10) was included in the Expanded Program of immunization (EPI) in Sindh, Pakistan in February 2013. PCV 10 is given as a three dose schedule at 6, 10 and 14 weeks of life with no catch-up currently offered. We undertook this study immediately before the introduction of PCV 10 to establish baseline pneumococcal carriage in naso-pharynx of children 3-12 months of age and 1 to 5 years of age in an urban community and children 3-12 months of age in a rural community in Sindh.

Methods & Materials: Baseline questionnaires were filled and nasopharyngeal specimens were collected from a random sample of children. Samples were processed in a central laboratory in Karachi using CDC standardized sequential multiplex PCR assay. Serotypes were then categorized into vaccine type and non-vaccine type.

Results: A total of 670 children were enrolled. Culture positivity rate for pneumococcus was 76 % and 80 % in the infant group in Karachi and Matiari respectively and 80% for children 1 to 5 years of age in Karachi. Prevalence of PCV 10 serotypes in infants was 30% and 23 % in Karachi and Matiari. In the older age group in Karachi, prevalence was 24%. Most common serotypes were 6A, 6B, 23F, 19A and 18C.

Table 1-Socio demographic and clinical characteristics of enrolled children

Pneumococcal carriage rates in Karachi and Matiari

Distribution of vaccine type and non-vaccine type serotypes in healthy children in Karachi and Matiari

Conclusion: This survey establishes the pre PCV 10 introduction vaccine and non-vaccine serotype carriage rate in children in a rural and urban community in Sindh. Annually planned surveys in the same communities will inform change in carriage rate after the introduction and uptake of PCV 10 in these communities.
Ex vivo evaluation of the mucoadhesive properties of Cedrela odorata and Khaya senegalensis gums with possible applications for veterinary vaccine delivery

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Background: The use of mucoadhesives in drug vehicle design has gained considerable attention over the past decades however, studies designed to evaluate the interaction of bioadhesives with biologic tissues with a view of application for non-invasive vaccine delivery in veterinary subjects' is scanty in literature. Hence, this study evaluates the peak adhesion time as well as capacity for vaccine delivery through the mucosal route of some phytogenic mucoadhesive polymers.

Methods & Materials: Gum gels from Cedrela odorata and Khaya senegalensis were harvested, purified, lyophilized and compressed into 500mg tablets individually and in combined ratios of 1:1, 1:3 respectively. These tablets were placed on freshly excised (about 5x5cm) trachea and duodenal tissues of cattle, chicken, pig, sheep and goat which were fastened to the basket end of a tablet dissolution machine probe. The probe set at 50rev/min was lowered into phosphate buffer at 6.8 pH in a beaker immersed in water bath at 37OC. The time it takes for the gum tablet to fall off the tissue under this condition is recorded as the peak adhesion time (PAT) of the gum polymer. The gum polymer with the best PAT was combined with Newcastle disease vaccine and the procedure repeated. Haemagglutination assay (HA) was conducted on the gum polymer-vaccine mix with gum and vaccine individually as controls.

Results: On cattle, chicken and sheep tissues, Cedrela-Khaya (1:1) mix had the highest PAT; goat, Cedrela-Khaya (1:3) mix while either Cedrela-Khaya (1:1) mix or Cedrela alone was best for pig tissues. On combination with vaccine, the PAT of the gums reduced slightly on cattle and sheep tissues while other animal tissue showed varied results. The HA results showed the gum polymer boosted the HA property of the vaccine (Log10^5), when compared to vaccine alone (Log 10^4). There was presence of HA property in the gum polymer alone. With a checkerboard dilution, the minimum dilution with the least HA property was recommended for vaccine dilution and in vivo application.

Conclusion: In conclusion, mucoadhesives from phytogenic sources has potentials for non-invasive vaccine application with possible amplification of duration of immune vaccine response.
Identification and characterization of a novel protein PfCDPK-5 for the development of pediatric malaria vaccine

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Background: Malaria are among the leading causes of mortality for children under five years of age worldwide, with most of these deaths resulting from *Plasmodium falciparum* infection. Resistance to existing anti-malarial medications is an urgent problem and may prevent effective eradication strategies. Despite decades of research, no vaccine candidate has been shown to confer significant protection to children. Though marginal protection has been achieved using the vaccine candidate RTS, S/AS01 and irradiated sporozoites, broadly effective vaccine candidates are urgently needed. The immediate goals of this study are to gain an immunological understanding of anti-PfCDPK-5 antibodies in preventing parasite maturation and egress.

Methods & Materials: In ongoing antigen discovery studies, we pioneered a high-throughput differential whole proteome screening method (phase display) to identify parasite epitopes associated with resistance to malaria in children. We utilized this approach to identify targets of antibodies that protect children from severe malaria or malaria-specific mortality and identified Schizont Egress Antigen-1 (Raj et. al Science 2014). In a parallel screening experiment, we used sera from 11 resistant and 14 susceptible children to differentially screen a parasite phage display cDNA library generated from parasite collected from African children. The localization study and growth inhibition activity were evaluated as per our published methods. Anti-rPfCDPK-5 antibody levels in the entire cohort measured by a bead-based assay.

Enrichment of antigens binding to malaria resistant kids antibodies

Results: We identified antibodies against PfCDPK-5 protein only in malaria resistant kids sera, not in susceptible kids sera. The preliminary data show that PfCDPK-5 phosphorylates PfSEA-1 another important protein responsible for egress of parasites. The polyclonal antibodies generated by recombinant PfCDPK-5 protein or DNA vaccine shows significant growth inhibition activity in *in vitro* assays. Anti-rPfCDPK-5 antibody levels in the entire cohort shows a positive correlation to morbidity and mortality of children.

Conclusion: In the present study, we validate a rationally identified vaccine candidate, *P. falciparum* calcium-dependent protein kinase 5(PfCDPK-5) using integrated translational approaches that harness high-throughput molecular techniques and *in vitro* functional assays.
Lesson learned from investigating cluster adverse event following immunization in mass campaign of Japanese Encephalitis vaccine in India

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Background: Vaccine safety is one of the critical parameters for quality assurance in immunization program in all countries including developing country like India as it adds new vaccines to its existing immunization program. Occurrence of adverse events following immunization (AEFI) and spread of unchecked rumors can hamper community confidence in vaccines adversely affecting coverage. Cluster AEFI (2 or more reports occurring together) get heightened attention from media, government and community and can affect performance of immunization program. In commitment to improve vaccine safety, this paper summarizes a report of a cluster investigation for AEFIs (90 reports) following Japanese Encephalitis (JE) vaccine given in a mass campaign in one district (Morigoan of Assam), India in June, 2014.

Methods & Materials: In response to received reports of AEFI cluster over 10 day’s period in June 2014, National AEFI surveillance team had investigated the reason for the events in the field by interviewing community and reviewing hospital records. Data of the individual cases was entered in anonymized line list and analyzed by using SPSS vs. 16. Results: In Morigoan, 200574 doses of JE vaccine was administered in 1-15 years age group during 15 days period in 2014 in mass campaign and among these 89 of the cases have reported symptoms of dizziness, tingling and numbness and abnormal movement of limbs. More than two- third of the affected individuals were females having median age of 9 years. Cases recovered without any residual sequel after receiving conservative treatment, reassurance and counselling in hospital.

Conclusion: There is a need of multi-pronged, effective information, education and communication intervention to handle unwanted rumors to ensure vaccine confidence during mass campaign by involving multiple stake holders.
The seroprevalence of neutralizing antibody against Japanese encephalitis virus in health care workers

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Background: Despite the introduction of inactivated Japanese encephalitis (JE) vaccine since 1990, JE remains an important cause of viral encephalitis in Thailand. Little is known about JE serostatus among Thais who were born before the 2001, the year that this vaccine has been included in the expanded immunization program. Therefore, the objective of this study was to determine the proportion of healthcare workers (HCWs) aged 21-60 years with adequate neutralizing antibody against JE virus.

Methods & Materials: We conducted a seroprevalence survey among HCWs during the routine annual check-up for HCWs at Queen Sirikit National Institute of Child Health (Children’s Hospital, Bangkok, Thailand) during the period between July-October 2015. A purposive sampling was done to enroll a relatively equal number of 4 different age ranges i.e., 21-30, 31-40, 41-50, and 51-60 years per each group. JE serostatus was determined using 50% Plaque Reduction Neutralization Test (PRNT). Immunity to JE were quantitated and cross-tabulated against age, gender, past and present domicile, history of JE vaccination, types of vaccine received (if any).

Results: A total of 400 HCWs among a total of 1,320 who received annual check-up were enrolled. Only 1.5% of participants reported having immunized with JE vaccine. 80.5% demonstrated an adequate existing antibody against JE virus genotype 3 Beijing strain (at least 10 reciprocal PRNT titer). The proportion of protective antibody (and corresponding geometric mean titer) of the 4 age groups were 77.0% (112.48), 82.4% (211.47), 80.6% (84.59), and 82.0%(126.97) among those aged 21-30, 31-40, 41-50, and 51-60, respectively. Male gender was the only parameter that significantly associated with the lack of protective JE antibody with risk ratio and 95% confidence interval of 1.69 (1.1, 2.7).

Conclusion: Since JE virus is unlikely to be transmitted in hospital settings, the result from this group might be reflective of those in the general adult population in Bangkok. As a result, approximately 20% of adult population in Bangkok may be at risk for acquiring JE when traveling to high risk/endemic area e.g. rural or upcountry.
Immunogenecity of a chimeric protein of Bacillus anthracis protective antigen and lethal factor in murine model

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Background: Anthrax, a disease of bioterrorism and public health importance is caused by the Gram positive, spore-forming bacterium, Bacillus anthracis. Anthrax toxin, a tripartite toxin is composed of protective antigen (PA), lethal factor (LF) or edema factor (EF). PA is the major protein which facilitates the entry of toxin component either of lethal factor or edema factor. Recombinant PA has been a suitable target for anthrax vaccine worldwide. However, instead of full PA, its domains are reported to provide protection. LF also contributes to immuno-protection against anthrax. Therefore, in this study, a chimeric protein consisting of both, PA and LF was developed as candidate vaccine for anthrax.

Methods & Materials: A chimera was made by fusion of immunodominant portion of PA (Domains 2-4) and LF (Domain 1) genes. The construct was cloned in pET32a+ vector and expressed in E. coli host. The recombinant chimeric protein was purified by immobilized metal affinity chromatography. The 4-6 week old Balb/c mice were injected intraperitonealy with three doses of chimeric protein (20 µg each mouse) at two week interval. The first dose was given with Freund's complete adjuvant and the subsequent doses were given with incomplete Freund's adjuvant. The serum IgG and its subtypes were determined by plate ELISA.

Results: The chimeric protein (PA-LF) was purified up to homogeneity and the production yield was 15 mg/l of the shake flask culture. The chimera elicited good immune response against both the toxins i.e. PA as well as LF. The end point titre of chimeric protein was 1:1024000 by plate ELISA. An antibody titre of 1:512000 was observed in mice serum for PA protein. The same serum exhibited the titre of 1:256000 against LF protein. The end point titres of IgG1, IgG2a, IgG2b and IgG3 were 1: 512000, 1:128000, 1:256000 and 1:32000, respectively. Thus, IgG1 was predominant among all subtypes indicating that PA-LF chimera induced Th2-type immune response.

Conclusion: The chimera consisting of partial sequences of PA and LF can be better vaccine candidate than individual PA or LF proteins. In the present study, the recombinant protein elicited very good immune response in mice and showed Th2 type of immune response.
Antibody response to various domain of protective antigen in cutaneous anthrax cases in India
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Background: Anthrax, caused by *Bacillus anthracis* is a well known biothreat disease. Besides, cutaneous anthrax is a public health disease also several countries where agriculture is the major source of income. Being a zoonotic disease, it primarily infects herbivorous livestock and wildlife species and then spreads to human through contact with infected animals or contaminated animal products. The virulence of *B. anthracis* is attributed to two major factors, i.e. a tripartite toxin and the poly-g-D-glutamic acid capsule. The anthrax toxins are secreted as three distinct proteins, namely protective antigen (PA), lethal factor (LF) and edema factor (EF) and their activities have been well described. PA is the pivotal protein of the anthrax toxin complex and therefore, has been a major target for vaccine development.

Methods & Materials: PA is a 83 kD protein which has 4 different domains. In this study, the 3 different domains of PA were cloned and expressed. The recombinant proteins were used to develop ELISA to determine the anti-PA IgG for individual domain in human cutaneous anthrax serum samples. End-point titers were defined as the highest serum dilutions that yielded an OD₄₅₀nm value 2-fold the value for the corresponding dilution of the control serum.

Results: Full PA protein (83 kD) and different domain proteins (PAD1, 46 kD; PAD2, 43 kD and PAD4, 33 kD) were purified to the homogeneity. A total of 41 cutaneous anthrax serum samples were examined for immunoreactivity with PA protein and its domains. The whole PA protein was found to give maximum immunoreactivity followed by domain 4, 2 and 1.

Conclusion: The immunoreactivity of human cutaneous serum samples with individual PA domains showed that besides full PA protein, individual domain 4 and 2 can also be good targets for vaccine development as well as for serodiagnostic assays.
Time trends in vaccine delivery over two decades in a full-time immunization clinic of a tertiary care centre

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Background: India’s national Expanded Programme on Immunization(EPI) launched in 1978 offers vaccines such as BCG, DPT, OPV, and measles vaccine free of cost, while pediatrician-recommended non-EPI vaccines are available for purchase in the country. On September 1st 1994 the Pediatric Department, Christian Medical College, Vellore expanded immunization services from 2 afternoons/week into a daily full-time walk-in immunization clinic offering both EPI and non-EPI vaccines. In this study we analyze time trends in vaccination provided by this clinic over two decades.

Methods & Materials: Manually entered records till 1st January 1996 and subsequent computerized clinic records were accessed for information on vaccine types and doses delivered from 1st January 1995 to 31st December 2014.

Results: The number of children attending the clinic showed a >2-fold increase from 31045 in 1995 to 89439 in 2014, averaging >7000/month and nearly 250/day in 2014. EPI vaccines increased proportionately for DPT (13313 in 1996 to 30641 doses in 2014), 4-fold for BCG(2650 in 1996 to 11610 in 2014) and over 5-fold for OPV(10452 in 1996 to 52200 in 2014). Hemophilus influenzae type b(Hib) vaccine was made available as a single vaccine from June 1997, as a quadrivalent(DPT-Hib) vaccine from September 1999 and as a pentavalent(DPT-Hepatitis B-Hib) vaccine from December 2002, nearly a decade before pentavalent vaccine provision on the national programme in 2011. Hib vaccine doses increased 6-fold from 4420 doses in 1998 to 28415 in 2014, with >15000 doses administered annually from 2001 and >20000 doses annually from 2008. The proportion of DPT and Hib administered as a combination vaccine increased from 3.7% in 1999 to 92% for DPT and 99% for Hib in 2014. Number of injections received at each child-visit did not exceed 2, and the average cost of a visit providing combination vaccines was kept to <500 INR(<10 USD).

Conclusion: Affordability and ease of access were the keys to sustained growth in vaccine provision from this private not-for-profit clinic of a tertiary care centre, and similar strategies can be used to improve immunization coverage in the country. Hib disease reduction in our community was documented by us earlier, attributable to Hib vaccine provision from this clinic.
Correlation of Interferon-gamma and Interleukin-28B levels in patients with chronic hepatitis C viral infection with or without Schistosoma mansoni coinfection
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Background: Hepatitis C viral infection (HCV) presents a serious health problem worldwide and is endemic in Egypt. Egypt has the highest prevalence of HCV in the world with an infection rate of one in five individuals. HCV infection in Egypt is usually associated with Schistosoma mansoni coinfection, another endemic disease in Egypt since the Pharos. Interleukin-28B (IL-28B) and interferon-gamma (IFN-g) are two of the mostly recognized immunological cytokines appearing in response to these diseases. The aim of our study was to investigate the relationship between IL-28B and endogenous IFN-g in untreated patients infected with HCV alone and coinfected with S. mansoni.

Methods & Materials: Serum levels of IFN-g and IL-28B were measured by ELISA in three groups: 50 untreated patients with chronic HCV infection (group I), 22 untreated patients with chronic HCV co-infected with S. mansoni (group II), and 35 healthy control subjects (group III). All patients were confirmed for HCV RNA positivity, with viral load quantitated by PCR. Routine liver function tests were performed for all groups, and diagnosis of S. mansoni infection was based on seropositivity for antischistosomal antibody by Indirect Haemagglutination technique.

Results: We found that serum total protein, albumin levels, and AST/ALT ratio were higher in HCV patients than in both HCV co-infected with S. mansoni and control groups (p<0.01). Meanwhile, g-GT levels were higher in HCV patients co-infected with S. mansoni than HCV alone (p<0.0001). IFN-g and IL-28B levels were directly correlated to viral load in both HCV and HCV co-infected with S. mansoni groups (p<0.001). Both serum IFN-g and IL-28B were significantly elevated in both chronic HCV patients and those co-infected with S. mansoni compared to controls (p<0.0001). Correlation analyses showed that IFN-g and IL-28B were positively correlated in patients with chronic HCV monoinfection (r=0.95, p<0.0001) and coinfected (r=0.87, p<0.0001) but not in control group.

Conclusion: In conclusion, our data suggests a strong positive correlation between IFN-γ and IL-28B in patients infected with chronic HCV with or without S. mansoni coinfection. Also, both cytokines are found to be associated with HCV viral load either with or without S. mansoni coinfection.
Outbreak investigation of suspected hepatities E among South Sudan refugees, Gambella regional state, Ethiopia, July 2014

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Background: Hepatitis E is a liver disease caused by the hepatitis E virus: it is a common cause of acute hepatitis with poor sanitation and hygiene. First week of June, 2014 Gambella regional state health bureau reported cluster of acute jaundice syndrome among South Sudan refugees living in Gambella regional state of Ethiopia. An investigation was conducted to identify the etiology of the outbreak, and to recommend control and prevention methods.

Methods & Materials: Patient observation and searching active cases was done in three refugee camps (Lietchuor, Kula 1 and Kula 2) and at MSF France clinic, line list and medical record also reviewed, 22 Serum specimens were tested for Hepatitis E by PCR techniques at CDC KEMRI/Kenya and screen for different vector-borne viral infections using IgM ELISA techniques at EPHI. Descriptive analysis was conducted by using Microsoft Excel.

Results: During May 4 – July 12, 2014 a total of 240 Jaundice cases were reported. 227 (95%) of the cases had fever, 238 (99%) of the total cases developed Jaundice. The overall median age is 23 years. From the total cases 99 (41%) were females and 141 (59%) were males, among those cases 6 pregnant women were found. There were 12 death of the 240 cases with a case fatality rate of 5 % 1 (8.3%) of whom was pregnant women. 200 (83.3%) refugees who develop Jaundice were reported from Lietchuor and 35 (14.5%) were from Kula 1 the rest 5 (2%) were from Kula 2. Of the 22 blood samples tested 12 (54%) were positive for Hepatitis E virus (HEV) by PCR technique.

Conclusion: The emergence of the outbreak in refugee camps is a major concern because of the associated difficulties in implementing effective preventive measures under camp conditions. Based on our findings the risk of the disease very high for pregnant women, the area was observed to be very prone to water born diseases, there was no latrine and shortage of safe drinking water is rampant. UNHCR and other partners initiated control measures, including health education on hygiene promotion activities, supplying safe drinking water and rushing to latrine construction.
Laboratory diagnosed dengue among clinically suspected febrile patient-samples at National Dengue Laboratory, Sri Lanka

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**Background:**
Dengue results with one or more of four related serotypes, DENV-1, -2, -3, and -4. Outbreaks hitting with different clinical manifestations spanning asymptomatic, classical dengue, dengue hemorrhagic fever and dengue shock syndrome. Different diagnostic tests are used to identify dengue different stages. This study based on laboratory-diagnosed dengue cases at National Dengue Laboratory at Medical Research Institute (MRI), Sri Lanka and it’s impact.

**Methods & Materials:** Study involved review of laboratory-diagnosed dengue cases for a one-year period beginning from March 2014. Reference laboratory received samples for testing from different hospitals across the country. Hospital visited, clinically suspected, both children and adult serum samples were tested using capture Dengue IgM ELISA assay, to detect anti-DENV IgM antibodies. Patients were diagnosed with recent infection if they were positive, followed by patient data investigation and comparison. Epidemiological data obtained from requests sent along with the samples.

**Results:**
This retrospective study investigated the positive cases for comparison of epidemiological data, clinical profile and outcome. Total 2779 were tested, 1493 (53.72%) were positive. 6% needed hospital intensive care and 1.6% received as post-mortem samples. 25% belonged to age 0-10 years and 2% were infants. Cases peaked in monsoon and post monsoon, May (65.56%), June (68.53%), and July (62.37%). 7.3% visited hospital in first five days, while 33% visited after day five of the illness. 1.7% associated with uncommon presentations; fits, chest-pain, cough, and urinary infection, 49% indicated common presentations; fever, myalgia, and headache.

**Conclusion:**
The study results confirmed the dengue threat in Sri Lanka and it’s huge impact on health-system. In order to target preventive measures effectively one should understand the dengue cases peaking during the monsoon and post monsoon seasons. Findings appreciated common and rare presentations of dengue and emphasized the importance of healthcare awareness of different clinical presentations and potential to improve laboratory diagnosis for early detection to make step forward in dengue management.
Measles among pregnant women in South Kazakhstan in recent times
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Background: In recent years, the measles incidence increase has been registered in many regions of Kazakhstan despite the fact that the national program of the infection elimination is in progress. There was its growth by 4.4 times in the Republic and by 2.2 times in the South-Kazakhstan oblast in the year 2014 compared to the year 2013.

Goals: comparative analysis of the clinical features and outcomes in pregnant women in South Kazakhstan.

Methods & Materials: One hundred medical cases of measles were analyzed among the in-door patients of the infectious disease hospital within the years 2014-2015, where adults made up 40 medical cases, children constituted 40 ones and pregnant women made up 20 ones with a different duration of gestation.

Results: Severe measles course was observed in 22.5% of medical cases in children, where it made up 45% in adults and constituted 100% in pregnant women. Fever (more than 38.5 degrees) was registered in children (72.5% of medical cases) made up 77.5% in adults and constituted 80% in pregnant women. Maculo-papular rash skinned out in 27.5% of pregnant women on the third day from the disease onset, it accounted 57.5% in pregnant ones on the 4th-5th day and made up 15% in ones being at a later date. Acute bronchitis (17.5%), pneumonia (7.5%), conjunctivitis (5%) and spontaneous miscarriage (8.3%) were among the complications in pregnant women. The delivery started in all pregnant women on the 2nd-3rd day of hospitalization being at the gestational age of 35-38 weeks (25%) and having acute toxicosis in the period of skin rashes.

Conclusion: Clinical course of measles is very severe in pregnant women with apparent toxicosis and some complications occurred. It is the triggering factor for spontaneous miscarriages and premature delivery.
An outbreak of measles in Ondo West LGA, Ondo State, Nigeria, February - May, 2013

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**Background:** Measles is a febrile rash illness and highly contagious viral disease associated with high morbidity and mortality. It is the fifth leading cause of death among under-five children worldwide. On 6th May, 2013, suspected measles cases were reported from Ondo West LGA to State Ministry of Health Akure. We investigated the outbreak to confirm diagnosis of measles, assess the magnitude, identify the source of the infection and institute control measures.

**Methods & Materials:** We conducted a descriptive study. We defined a case as any person under 5 years of age, residing in Ondo from 23rd February to 22nd May 2013, with history of fever and maculopapular generalized rash and cough, coryza or conjunctivitis or any person under 5 years of age in whom a clinician suspects measles. Suspected cases were line listed from the hospital register of Mother and Child Hospital, Ondo. Five (5) blood samples were collected from suspected cases and analyzed using enzyme linked immunosorbent assay (ELISA) technique. Data were analyzed using Microsoft Excel.

**Results:** 31 cases were identified. Mean age was 20 months. Out of 31 cases, 17 (54.8%) were males. The estimated population of Jilalu ward is 18,712 with under 5 children population of 3,742 resulting in an attack rate of 0.8/100,000 populations. There were 2 deaths reported (Case fatality rate: 6.4%). Males (54.8%) were more affected than females. Age distribution of children affected during the outbreak was: - less than 6 months (3.2%), 6 and 11 months (22.6%) and over 12 mths (74.2%). The first case was on 23rd February, followed by a rapid increase in the number of cases leading to a peak on 3rd May and a progressive decrease. The last reported case was on 22nd May, 2013. Immunization coverage for measles antigen in the affected local government between February-May 2013 was 85%, 65%, 38% and 60% respectively. Measles-specific immunoglobulin M (IgM) was detected by in three (60%) samples.

**Conclusion:** The low coverage of immunization might be identified as one of the key risk factor for the measles outbreak. Daily immunization services were commenced at the Mother and Child Hospital Ondo for children.
Assessment of Human Papillomavirus (HPV) type 16 and 18 status by nested multiplex PCR in cervical cancer patients and in healthy women visiting JIPMER, Pondicherry.

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Background: Cervical cancer is the most common malignancy in Indian women and approximately 130,000 women fall prey to cervical cancer every year. HPV DNA testing can be used as an adjunct to cytological screening of cervical samples of above 30 year old women and can be used as an optional test for follow-up in women who have slightly abnormal Pap test results. The aim of the study was to assess the HPV 16 and 18 status in cervical cancer patients and in healthy women visiting JIPMER, Pondicherry.

Methods & Materials: This study was approved by JIPMER Institute Ethics Committee. 171 biopsies from cervical cancer patients and 152 cytobrush samples from healthy women were collected. DNA was extracted using QIAamp DNA mini kit. The quality and quantity of the extracted DNA was determined by a spectrophotometer. The integrity of genomic DNA was checked by amplifying a 248-bp product of the human beta-globin housekeeping gene. A nested multiplex PCR was standardized and carried out to detect 2 high risk types, HPV 16 and HPV 18, further representative positive PCR amplicons were sequenced for confirmation.

Results: Out of 171 cervical cancer cases, 149 (87%) patient samples tested positive. Overall HPV-16 and 18 occurrence was found to be 87% in which HPV-16 was 64%, HPV 18 was 3% and mixed infection was 23%. Out of 152 healthy women cases, 78 samples were positive. Overall HPV-16 and 18 occurrence was found to be 51% in which HPV-16 was 22%, HPV 18 was 5% and mixed infection was 25%. Sequencing results were analyzed in NCBI BLAST to reconfirm the HPV genotype.

Conclusion: In our study, HPV-16 found to be the most common genotype in our region. Our study suggests that the HPV 16 and 18 mixed infection could be due to the consequence of poor genital hygiene and unsafe sexual behavior. In healthy women cases, it may be too early to come to a definitive conclusion about the HPV positivity because women under 30 who are sexually active are likely to have an HPV infection that will disappear on its own in future. But those women may need regular follow up.
Is HLA-DRB1*13 allele a risk factor for prognosis of hepatitis C virus infection?

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Background: Hepatitis C virus infection, with its high rate of chronicity, is a serious public health problem. There are studies being conducted on the factors affecting the prognosis of the disease. Changes of alleles in the human histocompatibility antigens (HLA) have been reported to affect the prognosis of HCV infection. In this study, we aimed to investigate the effects of HLA-DRB1 alleles on the treatment response and prognosis of the HCV infection.

Methods & Materials: This study was conducted with a total of 65 chronic HCV patients at Adnan Menderes University Medical Faculty Hospital. These patients were divided into three groups according to the prognosis of the disease: 1) patients who have responded to the treatment, 2) patients who has not responded to the treatment or had recurrence of the disease, 3) patients who have recovered spontaneously. Determination of HLA-DRB1 alleles were performed using sequence specific oligonucleotide (SSO)-Luminex method.

Results: The patients were in 22-79 years age range, and the average age was 56.5 ± 12.9. 43.1% of the patients (28) were male, and 56.9% (37) were female. Of the 65 patients with HCV infection in the study, 36 (55.4) responded to the treatment, 16 (24.6%) has not responded to the treatment or had recurrence of the disease, 13 (20%) have recovered spontaneously. The most common HLA-DRB1 alleles types found in patients were: HLA-DRB1*11 in 34 patients (26.15%), HLA-DRB1*4 in 20 patients (15.38%), HLA-DRB1*15 in 14 patients (10.17%), HLA-DRB1*13 in 13 patients (10%), HLA-DRB1*1 in 11 patients (8.46%). The patients who responded to treatment and patients who recovered spontaneously were found to carry the most HLA-DRB1*11 allele. The patients who have not responded to treatment or had recurrence of the disease were found to carry HLA-DRB1*13 allele the most. A statistically significant relationship (p <0.002) was found between frequency of HLA-DRB1*13 allele and the treatment or had recurrence of the disease were found to carry HLA-DRB1*13 allele the most. A statistically significant relationship (p <0.002) was found between frequency of HLA-DRB1*13 allele and the response to treatment and prognosis of the disease.

Conclusion: We concluded that carrying the HLA-DRB1*13 allele may be a risk factor for prognosis and response to treatment of HCV infection. Therefore, determination of HLA-DRB1 alleles may be necessary for effective treatment planning for patients with HCV infection.
Microbead array based technology for detection and quantitation of viral respiratory pathogens associated with pneumonia among children

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Background: Viruses play an important role in causing respiratory infections in children worldwide. The burden of viruses in respiratory infections among children in Pakistan is unknown, largely due to absence of good quality diagnostic facilities. Common viruses associated with respiratory infections included respiratory syncytial virus (RSV), influenza A/B, along with novel viruses like human metapneumovirus, human coronavirus NL63 and HKU1, and human bocavirus, which have the potential to cause pandemics. Therefore, it is, important to delimit the burden of viral pathogens in respiratory infections among children with viral pneumonia.

Advanced molecular biology techniques offer great advantage as they are more sensitive and have fast turnaround time for the identification of respiratory pathogens. Magpix platform is used to detect respiratory targets in multiplex assay based on the principle of magnetic bead which allows multiplexing of up to 50 unique assays in a single microplate well.

Methods & Materials: Nasopharyngeal swabs of children with acute respiratory infections were collected in viral transport medium and spiked with Bacteriophage MS2 extrinsic control to check the efficacy of nucleic acid amplification. An automated nucleic acid extraction was done using MagNa Pure instrument. xTAG Respiratory Viral Panel fast assay was used for the detection of wide range of viruses and subtypes. Data were analysed using TDAS RVP Fast software (ver. 2.21) and reported as median fluorescent intensity.

Results: Upon testing 734 nasopharyngeal swabs 362(49\%) were positive for entero/rhinovirus, followed by 48(6.5\%) positive for parainfluenza type III, 46(6.2\%) RSV, 38 (5.1\%) parainfluenza type IV, 35(4.7\%) metapneumovirus, 25 (3.4\%) bocavirus, 22 (3\%) adenovirus, 19(2.6\%) parainfluenza type I, 17 (2.3\%) coronavirus OC43, 16(2\%) influenza A/B, 7(1\%) parainfluenza type II and 5 (0.68\%) were positive for other coronaviruses 229E/ NL63/ HKU1.

Conclusion: Multiplex PCR- assay is rapid and sensitive tool for the detection of major respiratory pathogens and helpful in future for vaccine development. The advantage of this system is it has shortened time to perform a wide variety of bioassays, cost-effectively and accurately.
Seasonal drivers of WHO defined fast breathing pneumonia - impact of viral activity in the nasopharyngeal niche

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**Background:** The association between nasopharyngeal carriage of viruses and mild to moderate lower respiratory tract infections is poorly understood. We explore this association in an ongoing trial of fast breathing pneumonia with an objective to estimate the proportions of viral carriage in children with fast breathing pneumonia.

**Methods & Materials:** The study is being conducted in two primary health care centers in low income communities located in Karachi, Pakistan. Children 2 to 59 months old identified to have cough and tachypnea defined by WHO without danger signs and other illness like T.B., asthma, enteric illness are included. Viral carriages are assessed by taking nasopharyngeal swabs and are analyzed using LUMINEX xTAG Respiratory Viral Panel assay. The data has been collected for the period from September 2014 to August 2015.

**Results:** About 2126 (20%) of children in our community, who presented with respiratory symptoms at primary health center, are tachypnic. From these, 1055 are enrolled, nasopharyngeal results are available on 712 children. Rhinovirus is detected in 49% of the children, followed by Respiratory Syncytial Virus (RSV) in 6%, Human Metapneumovirus in 5% and Human Bocavirus and Adenovirus in 3% of the cases. Parainfluenza type III was detected in 7% and type IV in 5% of children, while rest of viral targets are found in ≤ 2% of the cases. The peak of Rhinovirus is found to be corresponding with increased presentation of cough in the months of October and January suggesting a positive epidemiological association. The rise of Human Metapneumovirus can be seen during early summer with peak in May and RSV in late summer (August to October) corresponding to increased episodes of fast breathing pneumonia. The peak of parainfluenza III coincides with increased episodes of tachypnea during January and February and Parainfluenza IV during December, March and May which seems to follow conventional seasonality pattern.

**Seasonal pattern of fast breathing pneumonia**

**Seasonality of fast breathing**

**Conclusion:** The variation and pattern in detection of virus among children is corresponding with symptoms of fast breathing pneumonia indicating their possible role in pathogenesis of the disease. This makes a case for exploring the role of antibiotics and conducting association studies with carriage rates in controls.
The curious cases of pandemic H1N1 pathology

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Background: The pathologic basis of pandemic H1N1 induced lung injury has been and still is an actively researched area. While findings from majority of the published literature suggest a rather general pattern of pulmonary pathology other reports have indicated the possibility of a typical pattern that still remains incompletely described. In India, the pandemic H1N1 reported cases were reported first in 2009. Retrospective analysis of archived tissue samples using light and electron microscopy including immunohistochemical analysis to examine the pulmonary pathology was the crux of the present study. A real time polymerase chain reaction for pH1N1 was used to detect viral sequences in autopsied tissue in fatal cases. Importantly, a unique aspect of this study was to compare several cases where the laboratory tests were inconclusive and examine whether a specific histopathologic lesion pattern typical to pH1N1 was observed.

Methods & Materials: Light and electron microscopy including immunohistochemical analysis to examine the pulmonary pathology was the crux of the present study. A real time polymerase chain reaction for pH1N1 was used to detect viral sequences in autopsied tissue in fatal cases.

Results: The main histopathological findings were diffuse alveolar damage (DAD) edema, hemorrhage, hyaline membrane formation, interstitial and septal edema with various degrees of mononuclear cell infiltration. There was minimal and focal chronic inflammation in tracheal and bronchial submucosa but cytopathic effect was seen in the form of irregularly enlarged hyperchromatic nuclei. Type 2 pneumocyte hyperplasia and alveolar septal fibroblast proliferation suggesting areas of transition towards organizing DAD was also observed (Figure 1 Panel A a-d). No microscopic features of vasculitis, thrombosis, hemophagocytosis or microorganisms were detected. Viral antigen was detected predominantly in the bronchial and bronchiolar epithelial cells, intra-alveolar macrophages, pneumocytes (Figure 1) and submucosal mucus-secreting glands of bronchus and trachea. Both nuclear as well as cytoplasmic positivity were noted. The rRT-PCR detected pH1N1 viral RNA in all 4 cases.

Figure showing representative microscopic lesions in lung and IHC for viral antigens

Conclusion: In summary, these findings suggests that the pH1N1 2009 virus infection could directly infect lower respiratory tissue, suppress immune response and may have a typical histopathologic pattern of lesions that could help in retrospective diagnosis of indeterminate cases.
Identification of occult hepatitis B virus (HBV) infection and viral antigens in healthcare workers who presented low to moderate levels of anti-HBs after HBV vaccination

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Background: Worldwide, healthcare workers (HCW)s show different levels of response to hepatitis B virus (HBV) vaccine. One of the factors associated with vaccine unresponsiveness may be the existence of current or past HBV infection. Regardless of the presence of HBsAg (overt infection), occult HBV infection (OBI, defined as presence of HBV DNA in the absence of HBsAg), might be also accounted in some non-or hypo-response cases.

Methods & Materials: Sera from 120 HBsAg negative HCWs with low and moderate levels of anti-HBs, <10 IU/mL (group 1) and <100 IU/mL (group 2) respectively, were selected and were examined for OBI by sensitive real time PCR regardless of HBV serological profiles. Direct sequencing on surface genes was carried out in OBI-positive cases.

Results: Four (3.3%) were positive for OBI. All were negative for anti-HBc. Two of the positive cases had moderate levels of anti-HBs (10-100 IU/mL) from group 2. No significant differences were found between the two groups in terms of risk factors or serological data. No mutations were found in surface proteins of OBI cases.

Conclusion: OBI in these subjects might be due to other factors rather than presence of “a” determinant mutations. Health care workers with inadequate to moderate levels of anti-HBs (<100 IU/mL) following vaccination, regardless of their serological profile for HBV, should be tested for the presence of HBV DNA by sensitive molecular tests. Anti-HBc is not a reliable marker for suspicion of OBI, especially in high-risk group individuals.
Sero-epidemiological investigation on enterovirus 71 among population in Chengdu, China


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**Background:** Hand, foot and mouth disease (HFMD) mainly caused by enterovirus 71 (EV71) infection has become one of the major public health issues in China. Understanding of population's immunity against EV71 and the epidemic changes of enterovirus are benefit to the future therapeutic and prophylactic intervention of HFMD.

**Methods & Materials:** Multistage stratified cluster sampling method was used for the sampling. A total of 623 patients infected with EV-71 during Jan and Dec, 2014 are selected from 3 representative city circles in Chengdu. The EV71-IgG levels of all the samples were detected by enzyme-linked immunosorbent assay. Statistical data comparisons between various factors were analyzed by means of Pearson $\chi^2$, unpaired T-test, or Fisher’s exact test. Correlation analysis is used to test the significance of the relationship between the positive rates and ages.

**Results:** It is of no statistical significance ($P > 0.05$) when it comes to the differences of the antibody positive rates of EV71-IgG between male and female, circles and circles as well as urban and rural areas. The positive rates of the groups with the age ranging 0~1 (61.37%), 1~2 (52.33%) are much higher than that of 3~4, 5~6, 7~9, 10~14, ≥15 groups, with the rates 29.89%, 22.52%, 14.54%, 11.26%, 7.33%, respectively (all $P < 0.05$). The positive rate remained relatively high in preschool children aged < 2 years and thereafter decreased sharply to more than 50% in individuals aged > 5 years. In terms of the correlation of positive rate of EV71 of the monitoring data and age of patients, it is negatively correlated when subjects are under the age of 5 while it turns to be positive when subjects are over the age of 5.

**Conclusion:** It is clearly identified that the population with the age under 5, especially the infants aged 2 years or younger, are the focus of the prevention and control of HFMD. Our study could play an important role in the protection of susceptible population and the evaluation of the immune effect of the upcoming vaccine application.
Circulation of dengue virus-1 Genotype III during 2015 dengue outbreak in Arunachal Pradesh: A maiden report from Northeast India  
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Background: Dengue is the most rapidly spreading arboviral disease in the world. Strain variations within the four dengue serotypes classify the virus into genetically distinct groups within serotypes called genotypes. Several studies have shown that disease outcome is associated with the genotype involved. Northeast Region of India comprises of 8 states and has witnessed several Dengue outbreaks in recent years. During 2015, a massive Dengue outbreak occurred in Pasighat, Arunachal Pradesh, India reporting around 2000 cases. All four serotypes have been reported from this region but studies on the genotype of the virus is lacking. This study was undertaken with an objective to elucidate the genotype of Dengue virus circulating in Arunachal Pradesh during the outbreak.

Methods & Materials: A total of 115 Dengue suspected samples were collected from Pasighat General Hospital, Arunachal Pradesh. Screening of Dengue NS1 antigen or Anti- Dengue IgM antibody was done depending upon the duration of sample collection and onset of symptom. IgG ELISA was done to determine whether the infection was primary or secondary. RT-PCR was performed using specific primers to amplify C-prM gene region. The obtained sequences were analyzed using BioEdit software and a phylogenetic tree was constructed in MEGA 6 software.

Results: Total 50 samples were Dengue positive by either NS1 or IgM ELISA of which eight (16%) cases were due to secondary infection. Dengue virus RNA was detected in 28 samples. Serotype 1 was found to be predominant during the outbreak. Phylogenetic analysis of the obtained sequences using Maximum Likelihood method and Kimura-2 parameter model revealed that the circulating Dengue virus-1 during the outbreak belonged to Genotype III.

Conclusion: This is the maiden report on genotyping of Dengue virus 1 from this region of India. Studies have shown that the emergence of newer dengue serotype/ genotype after a gap have always been associated with massive outbreaks; therefore it is necessary to carry out active surveillance to monitor Dengue virus epidemiology. Further study on pathogenecity of Dengue virus 1 as well as other serotypes circulating in this region is in scope of this study.
Cross-protective immunity against circulating Japanese encephalitis virus and West Nile Virus by live attenuated Japanese encephalitis vaccine SA 14-14-2

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Background: Co-circulation of Japanese encephalitis Virus (JEV) and West Nile Virus (WNV) has been accounted in India. Both viruses are antigenically related and belong to the Japanese encephalitis (JE) serocomplex. Recently, the Government of India introduced the live attenuated JE vaccine SA 14-14-2 in routine immunization program for children and mass vaccination campaign among adults in highly JE endemic areas of Assam, Northeast India. However, the protection elicited by the JE vaccine against the circulating JEV and WNV in this region have not been studied. Thus, we investigated whether a single dose of this vaccine provided protection against local JEV and WNV isolates in animal model.

Methods & Materials: Eight groups (n=6) of four-six week old Swiss albino mice were inoculated subcutaneously with the JE vaccine. Four weeks post immunization, three mice groups were challenged intraperitoneally with three JEV and four WNV each comprising of both archival and circulating strains. One mice group served as a control with no virus challenge. Mice were observed for 21 days.

Results: The protection rates against three JEV strains (genotype III) were 100%. However, we noticed limited protection against the four WNV stains (Lineage V). But, interestingly, the protection rates against archival WNV strains 804994 and circulating WNIRGC07 were 50% and 33.33% respectively. Whereas, no protection was conferred by the vaccine to WNV archival G22886 and circulating WNIRTC08 strains.

Conclusion: The study showed total protection against JEV strains which may be due to the same genotype of the vaccine (Genotype III) as that of the local JEV strains. However, JEV vaccine was found to elicit partial cross protection to a circulating WNV strain which was reported to be a variant. It is noteworthy that no protection was observed against the other circulating WNV strain. Thus for immunization strategies, limited cross protection against heterologous viruses of the JE serocomplex must be considered.
Minimisation study of dengue prognostic biomarker panel test

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Background: WHO has identified Dengue as the fastest spreading mosquito-borne disease in the world. Approximately 96 million people develop clinical Dengue annually, of which 1 in every 200 proceeds to develop potentially fatal Dengue haemorrhagic fever and/or shock syndrome (DHF/DSS). Depending on the availability of appropriate supportive treatment, the case-fatality rate varies from 3.5% to 50%. The debilitating and painful nature of the disease, together with the lack of an accurate method to predict DHF/DSS, resulted in unnecessary over-hospitalisation. The situation is worsened during epidemic years, in both developed (e.g. Singapore in 2004/2005) and developing countries (e.g. India in 2015) alike. Elective surgeries and non-emergency admissions had to be cancelled to release health resources for Dengue inpatients. Our laboratory previously discovered a panel of novel serum biomarkers that can predict DHF/DSS with a sensitivity and specificity of 90% and 91%, respectively. As majority of Dengue-endemic countries are developing countries, we sought to evaluate how test performance would be affected by using only one biomarker (BM1), so as to make the test more affordable.

Methods & Materials: 109 Dengue patients were enrolled from the Colombo South Teaching Hospital and National Hospital of Sri Lanka. The study was approved by the Ethics Review Committee of the University of Sri Jayawardenapura, and all patients provided informed consent. Blood samples were collected when subjects first presented themselves at the hospitals. Serum BM1 concentration was measured using a quantitative ELISA developed in-house. All statistical analyses were performed using R version 3.1.2.

Results: 60 subjects were diagnosed with DHF while 49 had classical Dengue fever. Based on only serum BM1 concentration, the performance of the test to predict DHF/DSS was as follows: sensitivity – 73.3%, specificity – 77.6%, positive predictive value (PPV) – 80.0%, and negative predictive value (NPV) – 70.4%.

Conclusion: While performance of the BM1 prognostic test (on Sri Lankan patients) could not match that of the panel prognostic test (on Singapore patients), there were considerable improvements in specificity and PPV over current clinical practices used (in Singapore) to determine which Dengue patients to hospitalise (specificity: ≤55%; PPV: ≤34%).
The efficacy and particular side effects of therapy peginterferon alpha-2a acute hepatitis C hemodialysed patients

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**Background:** Peginterferon alpha-2a is a known standard therapy for patients with acute HCV infection. Our experience with peginterferon in hemodialysed patients with hepatitis C started with a million question and uncertainties. This because of the:
- acute phase of infection, which we encountered due to repetitive serological tests the patients underwent because of hemodialysis protocols.
- fragile, immunodepressed patients which had a lifetime with serious underlying diseases, primary related to nephropathies or not.

However efficacy and safety of this treatment is still unclear in regional settings. This study tends to evidence the efficacy and safety of peg-interferon therapy in Albanian hemodialysed patients.

**Methods & Materials:** In a one-year period (from November 2013 - November 2014), we enrolled consecutive patients with detectable anti-HCV antibody and HCV-RNA in the serum, who had elevated serum alanine aminotransferase (ALT). Written informed consent was taken from them. Patients with decompensated cirrhosis were excluded and in women of fertility age, pregnancy tests were done 24 hours prior to the first dose of peginterferon alpha-2a. Underlying diseases in our hemodialysed patients included Chronic renal failure - 55 cases, Acute pyelonephritis - 1 case, Renal polycystosis – 6 cases, Congenital renal atrophy – 1 case, nephrolythiasis - 2 cases, renal transplant - 2 cases, nephrectomy- 3 cases, Arterial hypertension- 2 cases, spondyloarthrosis – 1 case.

**Results:** Early virologic response and sustained virologic response rates were 84.8 % (47/55) 78.2 % (42/55) respectively. The most common adverse effects in descending order were flue-like symptoms (83%), hair loss 36.3%, anorexia 54.5%, weight loss 25.4%, mood changes 40%, sleep disorders 29%, hematomas 41%, epistaxis 21.8% Laboratory data evidenced anemia 96.3%, leucopenia 78.1%, thrombocytopenia 83.6%.

**Conclusion:** Our 55 patients manifested several adverse effects, during therapy with peginterferon alpha-2a. Despite their particular immune status, these adverse effects appeared minimal compared to the efficacy of treatment in our patients.
The role of adenovirus 36 induced obesity in obese adults with cardiovascular disorders: The first clinical study investigating ad-36 antibody in sera and DNA in mediastinal adipose tissues of cases with cardiovascular disorders from Turkey (A preliminary study)

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Background: Recently, it has been showed that obesity strongly increase risk of morbidity and mortality caused by cardiovascular complications including atherosclerosis, cardiovascular disorders (CD), coronary heart disease. Obesity which develops due to multifactorial reasons, was associated with human Adenovirus -36(Ad-36). In this study, we aimed to investigate the role of adenovirus 36 induced obesity in CD.

Methods & Materials: In this cross-sectional and case-control based study, 75 obese(BMI ≥ 30 kg/m²) adults with cardiovascular problems, 28 non-obese(BMI ≤ 25 kg/m²) with cardiovascular problems, and also 48 people non-obese(BMI ≤ 25 kg/m²) without cardiovascular problem were included in this study as patient group(PG), patient control groups(PCG) and healthy control groups(HCG), respectively. For this purpose, mediastinal adipose tissue samples obtained PG and PCG from anterior mediastinum situated on the outer surface of pericardium during routine cardiovascular surgical procedures. Besides, the blood samples collected from the each groups(PG, PCG and HCG). In this preliminary study, the persence of Ad-36 antibodies, leptin, adiponectin levels were assessed by serum neutralization assay(SNA) and ELISA, respectively. Mediastinal adipose tissue samples will be examined for the presence of the Ad-36 DNA by PCR.

Results: Ad-36 antibody was detected in 10(13.3%) of 75 patients by SNA. We detected significantly difference Ad-36 antibody levels in the PG compared to the PCG and HCG(p<0.05). Mean BMI, leptin, LDL, triglyceride levels were higher in the PG, while adiponectin, levels were found to be lower in the PG. Significant differences were detected between the PG and PCG for the parametres(p<0.05), but there was no significant differences for total cholesterol.

Conclusion: In the light of our international literature review, although we detected a significant presence of Ad-36 antibodies related with obesity in adults with CD for the first time in this preliminary study, we planned to investigate the Ad-36 DNA in the mediastinal adipose tissue of obese adults, in order to demonstrate a possible Ad-36 relation clearly in obese adults with CD, once again for the first time in Turkey. There is a need for extended serial, particularly cohort and human-based, studies in order to have a clear understanding of the relation.
Factors leading to liver injury in acute dengue infection

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Background: Liver damage is commonly seen in dengue infection, which can sometimes lead to acute liver failure. Although the exact causes of liver injury is unknown, direct viral injury, hypoxic injury due to vascular leakage and immune mediated liver damage are thought to contribute to liver involvement in dengue. Therefore, we proceeded to investigate the patterns of liver injury and the possible contributing factors in acute dengue infection.

Methods & Materials: 55 adult patients with confirmed acute dengue infection were recruited during day 3 -5 of the illness and serial recordings of liver function tests, viral loads, serum IL10 and IL17 levels and the extent of fluid leakage were measured daily until discharge from hospital. According to the 2011 WHO guidelines, 19 of these patients were classified as dengue haemorrhagic fever (DHF) and 36 were classified as dengue fever (DF).

Results: Serum alanine transaminase (ALT), aspartate transaminase (AST), conjugated and unconjugated bilirubin, gamma glutamyl transaminase and alkaline phosphatase levels were highest on day 7 of illness in patients with DHF and DF. Serum albumin levels were only lower in patients with DHF. The peak in liver enzymes occurred 2 days after the peak of viraemia in patients with DHF and DF. The extent of the rise in liver enzymes did not correlate with the extent of vascular leak and there were no significant differences in any of the liver enzymes between patients with DF or DHF. In contrast, IL-17 levels were significantly associated with ALT levels (p=0.02, Spearmans r=0.17). IL-17 levels were significantly higher (p=0.008) on day 5 of illness in patients with ALT levels >4 times the upper limit of normal (mean 38.2 SE ±10.1), when compared to those with lesser degree of liver involvement (10.3, SE±10.2). Although IL-10 were higher in patients with higher AST levels, this was not significant.

Conclusion: Dengue associated liver injury appears to peak at day 7 of illness and appears to associate with serum IL-17 levels but not with the degree of fluid leakage or viraemia. Since IL17 was also shown to cause liver injury in dengue mice models, the mechanisms by which this occurs needs to be further investigated.
Molecular diversity of rotavirus strains from hospitalized children in Central Kerala

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Background: Group A Rotaviruses cause acute gastroenteritis (AGE) in children. In India, ~ 500,000 children are hospitalized with AGE annually, with an estimated 100,000 deaths attributed to rotaviruses. This study was aimed to characterize circulating rotavirus genotypes in a tertiary care centre in Central Kerala.

Methods & Materials: Stool samples (n=75) were collected from hospitalized children (age < 10 years) with symptoms of acute diarrhoea at Pushpagiri Institute of Medical Sciences and Research Center (PIMS & RC), Tiruvalla, Kerala between January 2013 - December 2013. Screening was done by rotavirus antigen detection ELISA (PremierTM Rotaclone,USA). Positives were confirmed by conventional Reverse Transcriptase based Polymerase Chain Reaction using published primers targeting VP6 gene. Genotyping was done by sequencing VP7 (G typing) and VP4 (P typing) genes, followed by phylogenetic analysis using MEGA.6 software.

Results: Of the 75 cases, 23 (30.6%) were positive for rotavirus by ELISA and RT - PCR. Among these positive cases, 26% required intensive care and three fourths of them were in 0 to 2 years of age . G1(n=17, 80.95%) was the most predominant G type detected, followed by G9 (n=4,19.04%) and few non-typeable strains (n=2,8.6%). P types were P[8(n=21,91.3%), P[6](n=1,4.3%) and P[4](n=1,4.3%). Phylogenetic analysis revealed that majority of G1 strains showed 98% homology with Indian strains and clustered in lineage 1,while few (n=3) clustered in lineage 2 with vaccine and other reference strains with a high bootstrap support. G9 strains exhibit maximum identity with Indian reference strains and were clustered in lineage 3. These strains showed only 87-89% identity with vaccine strain.
G1P8 (n=16,69.56%) was the most predominant strain circulating in this region. G9P[8], G1P[6], G9P[4] are the other strains encountered in this study.

Conclusion: This preliminary study helps to understand the rotavirus genotypes circulating in Central Kerala. Strains from this study clustered closely with previously reported Indian strains, indicating common ancestral strains. G9 and G1 strains showed only 87-90 % homology with vaccine strains, suggesting genetic diversity to escape from vaccine-derived immune response. This epidemiological data is important to detect the emergence of potentially epidemic strains, for the formulation of rotavirus vaccines.
High prevalence of hepatitis C and hepatitis B infection among pregnant women and their blood donors attending a surgical referral clinic in District Naushahro Feroze, Sindh, Pakistan 2014

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**Background:** Reports on burden of hepatitis C and hepatitis B virus (HCV/HBV) are a big challenge for district to minimize the transmission of associated risk factors. Study was conducted to know the prevalence of HCV/HBV infection in pregnant women and their blood donors attended a surgical referral clinic for cesarean delivery. Study was therefore undertaken to determine the prevalence of HCV/HBV infection among pregnant women and their blood donors.

**Methods & Materials:** A cross-sectional analytical study (record review) of all pregnant women and their blood donors (family members) was conducted from 10th to 15th February 2015 at a private surgical clinic in district Naushahro feroze. Data from 1st January to 31st December 2014 gathered on results of blood screening used by ELISA test.

**Results:** Of 175 women aged 15–52 years, 13%(n=22) tested positive (13 HCV; 9 HBV) besides these a total of 656 blood donors aged 16-40 years reported for blood donations and were 22%(n=145) tested positive (80 HCV; 65 HBV). The highest positivity rate was seen in women aged 25 years or less(26%) compared with those aged above 25 years (9%) OR=3.02 (95% CI 1.03 to 9.98). Statistically significant difference was identified between ages of donors who were aged below 25 years were high positivity rate(30%) compared to above 25 years positive for hepatitis infection(24%) OR=1.15.02 (95% CI 1.01 to 2.34).

**Conclusion:** One in eight pregnant women attending surgical clinic for cesarean delivery and one in five blood donors who came to attend for bleed has evidence of HCV/HBV infection. These HCV/HBV positive mothers may be at increased risk of transmitting HCV/HBV infection to their unborn babies. We suggest that all pregnant women attending 1st antenatal care be tested for HCV/HBV infection; exposed babies need to receive HBV vaccines at birth. Further molecular studies on risk factors should be carried out at these settings.
Significance of diagnostic kits evaluation for emerging dengue infection
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Background: Significance of diagnostic kits evaluation for emerging dengue infection
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Dengue is an arthropod borne flavi virus that includes of four serotypes (Den-1, Den-2, Den-3 and Den4) that comprises on multiple antigenic complex of family flavi viridae. Dengue virus infection causes range of clinical manifestations from asymptomatic and symptomatic as Dengue Fever (DF), Dengue Hemorrhagic fever (DHF) and Dengue Shock syndrome (DSS). Dengue cases are mostly asymptomatic fever with or without rashes. Within acute stage of infection, diagnostic confirmation is vital to recover the patients. The patients progress DHF/ DSS, which can result in death if it is not timely and managed appropriately. Although there are no specific antiviral drugs or vaccine for dengue infection, patient usually recovers with the appropriate management (fluids). Therefore, accurate and timely diagnosis and confirmation test have a key role in patient management and control. Many of the kits are commercially available for diagnosis of dengue infection.

Methods & Materials: The rapid detection technologies propose the guarantee fast and improved diagnostic for early detection and case management, but accuracy of the results has to be confirmed by evaluating the kit sensitivity, specificity and cross reactivity. Hence, the IgM, IgG, NS1 and Rapid immune chromatographic tests were evaluated and confirmed the results interpretations.

Results: The sensitivity of rapid immune chromatographic test was 97% and 91% specificity with 95% CI. Therefore this rapid diagnosis kit would be a first line diagnosis for dengue infection.

Conclusion: The rapid diagnostic kit is a promising technique to detect both primary and secondary dengue infections.
Rabies virus infection: Role of the rabies virus phosphoprotein in producing neuronal injury mediated by mitochondrial dysfunction and oxidative stress  

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Background: Our previous work in a mouse model of experimental rabies showed neuronal process degeneration in association with severe clinical disease. Cultured adult rodent dorsal root ganglion neurons infected with the challenge virus standard-11 (CVS) strain of rabies virus (RABV) showed axonal swellings and reduced axonal growth with evidence of oxidative stress. We have shown that CVS infection results in increased reactive oxygen species (ROS) production and mitochondrial Complex I activity. The RABV phosphoprotein (P) was detected by immunoblotting in RABV-infected purified mitochondrial extracts from mouse neuroblastoma cells and in Complex I immunoprecipitates from the extracts. A plasmid expressing P in cells increased Complex I activity and increased ROS generation, whereas expression of other RABV proteins did not. Expression of a peptide from amino acid 139-172 of P increased Complex I activity and ROS generation similar to expression of the entire P protein, whereas peptides that did not contain this region did not.

Methods & Materials: Mutational analyses were performed to evaluate the role of the RABV P in Complex I activity and ROS generation.

Results: We performed alanine mutagenesis of overlapping triplicate adjacent sites over the 139-172 P region and on seven conserved amino acids and mutagenesis to both alanine and aspartate on four serine residues in this region. Mutational analysis suggests importance of the 145-151 and 157 to 169 regions of P. Three (159, 162, 166) of four serine residues were important and double alanine mutations had greater effects on activities. Six (144, 146, 147, 155, 167, 170) of seven conserved amino acids were also important.

Conclusion: A region of the RABV P interacts with Complex I in mitochondria causing mitochondrial dysfunction, increased generation of ROS, and oxidative stress. Therefore the RABV P plays a key role in the induction of mitochondrial dysfunction and generation of ROS resulting in oxidative stress in rabies virus infection through an interaction with Complex I. The resulting mitochondrial dysfunction produces oxidative stress in neurons that causes acute degenerative changes affecting neuronal processes resulting in a severe and fatal clinical disease. This information will be important for the future development of novel therapies for rabies.
Dengue 2 virus infection associated vascular endothelial cellular stress response imaged by high resolution electron and correlative microscopy shows distinct evidence of altered cytoskeleton and vesicular traffic. A. Basu, D. P. Jain

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Background: Dengue virus (DENV) is an enveloped Flavivirus that is of significant global public health importance. The virus is transmitted through bite of infected Aedes egyptii mosquitoes and can cause clinical disease in humans ranging from mild asymptomatic subclinical infections to severe life threatening forms called dengue hemorrhagic fever and shock syndrome (DHF/DSS). The pathogenesis of dengue virus infection remains incompletely understood. Endothelial cells have been shown to be natural targets of dengue viruses and dysfunctional responses of these cells cause the severe hematological crisis of DHF. The nature of cellular stress of the vascular endothelium to DENV remains unknown. In the present study we used a correlative integrated high resolution microscopy approach (CLEM) to image the nature of fine structural changes in DENV 2 infected endothelial cells of hepatic sinusoidal origin.

Methods & Materials: DENV 2 infected cells were observed through early and late time point scales and virus replication detected through immunofluorescence and polymerase chain reaction assays. Infected cells were harvested and observed in three different microscopy platforms for high resolution imaging. Atomic Force Microscopy on live cells, confocal microscopy for tracking viral antigens and cryosubstituted cells imaged under high resolution transmission electron microscopy and tomography for fine structural changes.

Results: The integrated CLEM observations revealed early changes in vesicular traffic; actin depolymerization; autophagic response; cisternal dilatation of the endoplasmic reticulum and changes in cell surface caveolar morphology. Moreover, a very unique observation was co-localization of virus with cellular mitochondria but absence of apoptotic morphology.

Conclusion: Taken together, these findings, for the first time provides insight into fine structure of endothelial cellular stress response suggesting that basic physiological alteration in actin dynamics and signaling pathways are fundamental in endothelial dysfuction directly affected by the virus. Elucidation of this host pathway has potential for developing novel drug targets.
Expansion of regulatory T cells in acute dengue infection does not associate with disease severity


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Background: Regulatory T cells (Tregs) with suppressive function were shown to expand in acute dengue and were speculated to contribute to milder disease. However, as more recent data point towards a protective role of dengue virus (DENV) specific T cells in acute dengue, we proceeded to determine if the expansion of Tregs in acute dengue was associated with milder clinical disease.

Methods & Materials: 58 adult patients with acute dengue infection were recruited and disease severity classified according to 2011 WHO guidelines. Convalescent samples were obtained from 10 of these patients 30 days after onset of illness. Tregs were identified in both patients and in 13 healthy individuals by staining for CD4+ T cells expressing Foxp3 and CD25. Quantitative ELISA was done to determine plasma levels of IL-10, TGFβ and IL-17. Intracellular cytokine staining for IL-17 was carried out and TH17 subset of CD4+ T cells was identified as those expressing, IL-17 and CD161.

Results: Tregs were significantly expanded in patients with acute dengue (p<0.0001) when compared to healthy individuals and the frequency of Tregs significantly reduced during convalescence (p=0.01). The frequency of Tregs were not significantly higher in those with milder forms of dengue and did not associate with the extent of fluid leakage, presence of shock or liver derangements. The frequency of Tregs in acute dengue, did not correlate with either plasma levels of IL-10 or TGFβ. Expression of CD25, which is the IL-2 receptor, on CD4+ T cells was significantly lower (p=0.006) in patients with acute dengue and CD25 expression inversely correlated with IL-10 and TGFβ levels. Both plasma IL-10 (p<0.0001) and TGFβ levels (p<0.0001) were significantly elevated. However, no difference in plasma IL17 levels were observed and the frequency of TH17 subset of CD4+ T cells and Treg: TH17 ratios were similar in both patients and healthy individuals.

Conclusion: Although Tregs are increased in frequency during acute dengue, they do not appear to associate with milder clinical disease. Immunosuppressive cytokines (IL-10 and TGFβ) were significantly elevated in patients with acute dengue and inversely correlated with CD25, which suggests that they possibly suppress T cell proliferation.
Obesity and the presence of asthma are associated with hospitalization due to dengue infection
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**Background:** Although dengue infections can lead to severe clinical disease sometimes resulting in fatalities, the majority of both primary and secondary dengue infections result in mild/asymptomatic disease that is usually not diagnosed as dengue. Therefore, we proceeded to investigate epidemiological and co-morbid risk factors associated with hospitalization when infected with dengue.

**Methods & Materials:** 1689 healthy individuals who were attending the primary health care facility of the university were recruited. Information regarding their co-morbid illnesses and anthropometric measurements were recorded. The dengue antibody status was determined in all individuals.

**Results:** Although 1152/1689 (68.2%) individuals were seropositive for dengue and only 133/1152 (11.5%) of them had been hospitalized due to dengue. We found that obesity (BMI > 22.9 in adults and above the 85th percentile BMI for age for children), asthma, allergic rhinitis and a waist circumference of > 80cm in women was significantly associated with increased risk of hospitalization. The association of hospitalization due to dengue and obesity was stronger for females (P < 0.0001, odds ratio = 3.33, 95% CI = 1.8 to 6.1), when compared to males (p value = 0.04, odds ratio = 2.2, 95% CI = 1.1 to 4.5). Although female children were significantly more likely (p = 0.006) to be hospitalized due to dengue (odds ratio 2.4, 95% CI = 1.3 to 4.4) when compared to male children, no such association was observed in adults. The presence of diabetes, hypercholesterolaemia or hypertension were not significantly associated with hospitalization due to dengue.

**Conclusion:** Obesity, a high waist circumference in women, asthma and allergic rhinitis appear to be associated with a higher risk of hospitalization when infected with the dengue virus.
Identification of viral and immunological correlates of disease severity and recovery in pediatric dengue patients

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Background: Dengue infection manifests as a wide range of symptoms, from a mild fever to fatal dengue shock syndrome. The presence of four serotypes and infection-enhancing antibodies pose a challenge to further investigate the role of virus and immune response in dengue pathogenesis. The serotype of the infecting virus, viremia and a number of inflammatory mediators have been identified as risk factors in severe dengue disease but a comprehensive analysis of these factors in an Indian cohort has not been reported.

Methods & Materials: We evaluated the viral and immunological factors that correlate with severe dengue disease in a cohort of pediatric dengue patients in New Delhi. Dengue-infected patients were enrolled and classified into three disease severities namely, Dengue Infection (DI), Dengue with Warning signs (DW) and Severe Dengue (SD) based on WHO classification. Blood samples were collected and the infecting virus serotype and viremia were analysed. Plasma cytokine levels were estimated by multiplex magnetic-bead assays. Peripheral blood mononuclear cells were isolated and dengue positive cells were identified by staining cell surface markers and viral antigen and analysis by fluorescence activated cell sorting technique. Markers of disease severity and recovery were identified by computational approaches involving multivariate analysis.

Results: Severe dengue disease was observed in both primary and secondary infections. Viral load had no association with disease severity but high viral load correlated with prolonged thrombocytopenia and delayed recovery. Severe dengue cases had low Th1 cytokines and a concurrent increase in the inflammatory mediators such as IL-6, IL-8 and IL-10. A transient increase in CD14⁺CD16⁻ intermediate monocytes was observed early in infection. The CD14⁺ cells, but not the CD16⁺ or the T or B cells, were positive for dengue antigen and were major producers of IL-10. Reduced interferon-α levels and enhanced pro-inflammatory cytokines were identified as some of the distinctive markers of severe dengue. Furthermore, the cytokines IL-8 and IL-10 were identified as the most significant markers of recovery from severe disease.

Conclusion: Our results provide further insights into the immune response of children to primary and secondary dengue infection and help us to understand the complex interplay between the intrinsic factors in dengue pathogenesis.
In-vitro problems of screening (Anti-HCV) and confirmatory tests (RIBA) for the diagnosis of HCV infections: The relation of neopterin and sCD14 with low Anti-HCV reactivity and different RIBA patterns

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Background: Neopterin and sCD14 levels could be detected at high levels in HBV, HCV and HIV infections as well as pathologies including trauma, sepsis and rheumaoid arthritist. We aimed to evaluate the importance of neopterin and sCD14 levels for the determination of HCV infection in situations where low Anti-HCV reactivity and indeterminate RIBA are present. We also aimed to compose a new diagnostic perspective for the atypical cases which have low Anti-HCV reactivity and different RIBA results

Methods & Materials: As Total Study Group (TSG), 70 individuals with low reactive Anti-HCV test results due to >1-<3,8 signal/cut-off value and with 30 negative, 30 indeterminate and 10 positive RIBA results were included. As Patient Control Group (PCG), and Healthy Control Group (HCG), thirty individuals per group were included. ELISA method, HCV RIBA/LIA and HCV-RNA tests were used. Neopterin and sCD14 levels were measured by competitive and serological EIA methods

Results: When groups were compared for neopterin levels; mean neopterin level was found low with advanced significant difference in TSG when compared with PCG, (p<0.001). On the other hand, no significant difference was found between TSG and HCG (p>0.05), as the levels were close each other. Mean sCD14 level was found significantly higher in TSG than PCG and HCG (p<0.005, p<0.001)

Conclusion: Neopterin levels determined in the cases with low reactive Anti-HCV and different RIBA patterns could not suggest a specific immunoactivation based on HCV infections. We think that sCD14 test may probably have been non-specifically affected as a result of some underlying immunohematological atypical pathologies. Therefore, the results of this study suggest that using only neopterin not sCD14, together with fourth-generation Combo tests based on EIA/CMIA will be useful in the situations where NAT can not be available for screening of blood donors in blood banks
Influenza illness in pregnant Indian women: A cross sectional study

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Background: Pregnant women were observed to be at high risk of complications such as pneumonia and death during the influenza pandemics of 1918, 1957, and 2009 and accounted for about 6% of influenza related hospitalizations ICU admissions and deaths in the 2009 influenza pandemic in the USA. Data on burden of influenza in pregnancy in India are scant. We set out to study the contribution of influenza viruses in the causation of respiratory illness during pregnancy in a north Indian setting with temperate seasonality of influenza.

Methods & Materials: A total of 266 pregnant women (> 18 years) presenting with influenza like illness (ILI) or severe acute respiratory infection (SARI) were enrolled from December 2014 till May 2015. Clinical details of the patients were recorded and twin nasal and throat swabs obtained. Samples were tested by real-time RT-PCR for influenza viruses A and B using the standard CDC protocol and sub typing for influenza A positive samples for A/H1N1p09 and A/H3N2 was done using specific primers. Influenza B viruses were subtyped into B/Victoria and B/Yamagata lineages using specific primers.

Results: Of the 266 pregnant females (age 17-39 years; median 27 years), 50 (18.8%) were detected positive for influenza (A/H1N1pdm09 = 41, A/H3N2 = 8 and B/Yamagata = 1). Rigors (96%) and headache (90%), history of ARTI (40%) and ILI (4%) in the family were seen significantly more (P<0.05) in influenza positive females in comparison to influenza negative ones (table 1). Nine (18%) participants required admission for their illness; 5 developed respiratory failure and 4 developed pneumonia. Oseltamivir was given in all influenza positive cases along with supportive therapy (including ventilation). Four of the patients (3 in third trimester) died whereas others had an uneventful recovery.

Clinical features in influenza positive and influenza negative participants

Conclusion: Influenza viruses causes acute respiratory illness in a significant proportion of pregnant Indian females resulting in considerable morbidity and mortality. These, first data from the country, call for strategies aimed at reducing influenza burden in pregnancy like vaccination alongwith institution of early antiviral therapy.
Viral etiology of anterior uveitis in immunocompetent patients in North India

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Background: Uveitis is the leading cause of blindness worldwide. Among various forms, the anterior uveitis is most common. Herpes simplex virus (HSV) and varicella zoster virus (VZV) are known to cause anterior uveitis. However, recent studies have also reported the role of human cytomegalovirus (HCMV) in the causation of anterior uveitis in immunocompetent individuals. The present study aimed to study the prevalence of HSV-1, VZV and HCMV in patients presenting with anterior uveitis in a tertiary care hospital in North India.

Methods & Materials: A total of 41 aqueous humor samples were obtained from the patients with anterior uveitis. The samples were subjected to DNA extraction followed by real time PCR using primers and probes targeting gD of HSV-1, ORF28 of VZV and IE gene of HCMV.

Results: The mean age of the patients were 45.2 ± 13.03 years. The male:female ratio was 1.8:1. Redness, blurring of vision and pain was observed in 78% (32/41), 73.2% (30/41) and 46.3% (19/41) of the cases. The viral etiology was attributed in 30.2% (13/43) of patients. HSV-1, VZV and HCMV DNA were detected in 7(17%), 7 (17%) and 1 (2.5%) of patients respectively. The mean viral load for HSV-1 and VZV was found to 9.4X10^4 copies/µl and 1.6X10^5copies/µl respectively. The 3.2X10^4 copies/µl of load was found in HCMV positive patient. Viral uveitis patients presented with raised intraocular pressure (57%-60%), keratic precipitates (60%-71.4%) and iris atrophy (42.8%-60%). The majority of cases with viral uveitis had disease duration of ≤6 months (60%-71.4%).

Conclusion: HSV-1 and VZV are the important causative agents of viral anterior uveitis. HCMV as an infectious cause for anterior uveitis is rare among immunocompetent patients in North India. Real time PCR is a sensitive and specific technique for the early diagnosis of viral uveitis which in turn will help in the early initiation of therapy thereby preventing irreversible loss of vision.
Real time PCR for the diagnosis of Rubella Virus, Herpes Simplex virus-1 and Toxoplasma gondii in patients with congenital cataract

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Background: Congenital cataract is most serious type of cataract and has the potential for inhibiting early visual development. Intrauterine infections with Rubella virus (RV), Herpes simplex virus-1 (HSV) and Toxoplasma gondii play an important role in the development of congenital cataract. The aim of present study was to know the contribution of RV, HSV-1 and T.gondii towards congenital cataract in children attending a tertiary care hospital in North India.

Methods & Materials: The study included 120 children under the age of 6 years presenting with congenital cataract. The blood samples and lens aspirates were collected from patients undergoing cataract surgery i.e phacoaspiration with or without intraocular lens (IOL) implantation under general anesthesia and subjected to serology and real time PCR. The real time PCR was performed targeting E1 gene of RV, gpD gene of HSV-1 and B1 gene of T.gondii.

Results: The male: female ratio was 1.7:1 with age range from 2- 72 months. Majority (88.3%, 106/120) of the patients had bilateral cataract. The predominant type of cataract was zonular (50.8%). The IgM positivity for RV, HSV and T.gondii was found to be 5.8%, 1.6% and 8.3% respectively. The real time PCR positivity was found to be 21 (17.5%), 19 (15.8%) and 32 (26.6%) for RV, HSV-1 and T.gondii respectively. The mean viral load for rubella, HSV-1 and T.gondii was found to be 1599 copies/µl; 1716 copies/µl and 1503copies/µl. Considering serology and real time PCR, mixed infections were observed in 16 (13.3%) children tested.

Conclusion: The serology has a limited role in the diagnosis of congenital cataract. Infective etiology significantly contributes to the causation of congenital cataract particularly for RV which is a potentially eradicable disease. This study provides an epidemiological data for rubella, HSV-1 and T.gondii in children with congenital cataract and highlights the need to introduce rubella vaccine in the National Immunization Programme of India.
Hepatitis A outbreak associated with unsafe drinking water in a medical college student’s hostel, New Delhi, India, 2014

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Background: An outbreak of jaundice was reported from a girl’s hostel (Old Girl’s Hostel; OGH) of Maulana Azad Medical College (MAMC), Delhi in January-2014. We conducted an investigation to identify the etiological agent, source of infection, potential risk factors and to recommend control measures.

Methods & Materials: We conducted a retrospective cohort study of all OGH residents. We defined a case as illness in any person residing in OGH, who reported jaundice with ≥ 1 of the following symptoms: nausea, vomiting, diarrhea, fever, anorexia, or abdominal pain between 15th-August-2013 to 21st-February-2014. Data on potential exposures was collected using a pretested structured questionnaire and relative risks were calculated. Laboratory confirmation for acute viral hepatitis-A (IgM) and hepatitis-E (IgM) was conducted on case-patients using ELISA. Water specimens from end-points of water supply-lines were tested for fecal-coliforms using most probable number (MPN) method.

Results: All 226 residents of OGH were female students, median age 20 years (range=17-22); 28(12.2%) met the case definition. No clustering of cases was observed near any specific water source or sanitary facility of the hostel. Those who drank from water coolers of the hostel kitchen were more likely to be cases than those who did not (96% vs 29%; Relative Risk-3.3, 95% Confidence Interval: 2.5-4.4). Of 11 specimens laboratory tested, 7 confirmed positive for Hepatitis-A. Onsite inspections revealed that the water-pipeline supplying the mess cooler was damaged. Among 41 water specimens from taps supplied by this pipeline, 17 demonstrated fecal-coliforms (median-17/100ml).

Conclusion: Damaged water supply pipeline may have contaminated drinking water and resulted in this water-borne hepatitis-A outbreak at an urban location. The water-pipeline was replaced leading to cessation of the outbreak. Regular monitoring of quality of drinking water was instituted.
Immature platelet fraction in Dengue cases
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**Background:** Immature Platelet Fraction (IPF) is an index of thrombopoiesis which quantitates reticulated platelets that have been recently released from the bone marrow.

**Objectives:**
To find out the association between the status of immature platelet fraction (IPF) and the recovery of platelets in patients with dengue.

**Methods & Materials:** A prospective study was designed done to find out the association between the status of immature platelet fraction (IPF) and the recovery of platelets in 45 Dengue confirmed (positive NS1 or IgM antibody dengue test) cases whose platelet count was less than one lakh/cumm with or without a downward trend. Platelet count and IPF were estimated using Sysmex XE-2100 (Sysmex, Kobe, Japan). Complete blood count was recorded simultaneously and peripheral smears were studied in all these cases with a note for the presence of large platelets on smear. The cases were managed conservatively. The work was carried out after an approval from Institutional ethics committee. Data was analysed statistically.

**Results:** Among the recovery of platelets, 86.4% showed recovery within 24hrs and the rest with 48 hrs after attaining peak IPF value. A single value IPF more than 10% was indicative of platelet recovery within 24-48 Hours. A positive correlation was observed among immature platelet fraction (IPF) level and the recovery of platelets in those patients with dengue.

**Conclusion:** IPF had a positive correlation with recovery of platelet counts in patients with dengue infections. Hence, Practitioners handling Dengue cases may be oriented to look for IPF, and consider it before referral or active intervention.
Molecular detection and characterization of Sapovirus from hospitalized cases of acute gastroenteritis from western India

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Background: Acute Gastroenteritis (AGE) accounts for 2.5-3 million deaths per year in children below 5 years of age. Among enteric viruses, Rotavirus is the leading cause of this diarrhoeal disease, followed by Norovirus, Adenovirus, Astrovirus and Aichivirus. Sapovirus (SaV), members of the Caliciviridae family are known to cause outbreaks and sporadic cases of AGE. SaVs are classified into 5 genogroups: GI-V, based on nucleotide variation in their capsid region. Among the 5 genogroups, GI, GII, GIV and GV are known to infect humans while GIII infect porcine and mink hosts. In India, studies on SaVs associated with AGE are available from northern, eastern and southern regions; however, no such data is available from Western India.

Methods & Materials: Stool samples of children ≤5 years of age (n=418), hospitalized for acute gastroenteritis; collected from Pune, Maharashtra, western India, between Jan 2009-Dec 2011 were included in the study. Detection and genotypic characterization of SaVs was carried out by amplification of the RdRp-Capsid junction region (~420bp). The amplicons were sequenced using ABI Prism ABI3730XL automated sequencer. Phylogenetic analysis of sequences was performed using MEGA 6 computational tool.

Results: SaVs were detected at an overall prevalence rate of 2.4% (10/418) in AGE cases. Co-infection with Astrovirus, Enterovirus and Adenovirus were observed in 3/10 cases (30%). SaV infections were observed in children ≤3 years of age, mostly in summer (60%) and monsoon (30%) with peak SaV activity reported in March (50%). Severity assessment of AGE revealed mild (20%), moderate (40%) and severe (40%) infection in SaV positive cases. Phylogenetic analysis of study strains revealed the circulation of GGI (10%), GGII (50%) and GGV (40%) in the study region. GGI strain showed highest nucleotide identity with GGI strains from UK (100%), while GGII and GGV strains showed nucleotide identities ranging between 96.8%-99.6% and 99.2%-99.6% with their respective strains from UK, Thailand, Australia, Japan and USA.

Conclusion: The study reports circulation of GGV strains of SaV in AGE for the first time in India and also sheds light on the genotypic distribution of SaVs in Western India.
Identification of human papillomavirus types causing lesions in penile canerous, pre-cancerous and benign lesions using laser microdissection

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Background: Almost half of penile cancers and the majority of penile warts are associated with the two HR types (HPV 16 and HPV 18) and two LR HPV types (HPV 6 and HPV 11), respectively. It is therefore important to document the burden HPV associated diseases of the penis and identify the HPV types associated with the diseases. HPV E6 and E7 mRNA detection is the best indicator of HPV status that is clinically relevant as it indicate transcriptionally active HR HPV infection in lesions. In HR HPV infection an increase in E6 and E7 mRNA expression causes an overexpression of p16INK4A and thus used as a cellular correlate of the increased expression of HR HPV E6 and E7 mRNA in cervical samples. The aim of the study was to identify HPV type responsible for the development of the penile lesions.

Methods & Materials: To do that we genotyped and quantified 66 (18 benign lesions, 4 pre-cancerous and 44 cancerous lesions) penile tissue biopsies and performed LMD on the selected penile samples (7). RNA in situ hybridization (ISH) was performed using HPV16 alone, for HPV HR18 cocktail: HPV types 16,18,26,31,33,35,39,45,51,52,53,56,58,59,66,68,73,82) and HPV11 probes.

Results: HPV 11 (50.9%) and HPV 16 (49.1%) showed almost similar incidence in the study patients. Multiple infections were observed in 18/55 (32.7%) of the positive samples, majority were in condyloma and verrucous carcinoma cases and HPV 11 showed higher viral loads compared to the other HPV types. After lesion dissection with LMD and HPV 11 and/or HPV 16 were the only types detected, compared to multiple HPV types (HPV 11, HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, and HPV 39) initially detected in whole tissue sections. Almost all the SCC lesions were positive for HPV 16 and showed p16INK4A overexpression in contrast there was no expression of p16INK4a in verrucous carcinoma and condyloma acuminatum lesions irrespective of the HR HPV types present.

Conclusion: In conclusion p16INK4a overexpression and mRNA expression correlated with HPV type associated with the lesion.
Viral aetiologies of acute encephalitis in a hospital-based population in Sri Lanka
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**Background:** The aetiological spectrum of acute encephalitis in Sri Lanka remains unknown. We aimed to identify the viruses which are known to be a major cause of infectious encephalitis

**Methods & Materials:** A cross-sectional study was conducted among 99 patients with encephalitis/meningoencephalitis admitted to two tertiary care hospitals in Colombo. CSF and serum were tested for conventional viruses and emerging viruses that can cause encephalitis. Specific nucleic acid amplification assays and antibody assays were used to identify viruses. Plaque reduction neutralization test (PRNT) was done to confirm the diagnosis of West Nile virus (WNV).

**Results:** Patients’ age ranged from 1 month to 73 years (mean=24.91; SD=21.33) with male: female ratio of 1.75:1. A viral aetiology was identified in only 27.3 %. These included Dengue virus (40.7%), Japanese encephalitis virus (25.9%), Varicella zoster virus, Epstein Barr virus and WNV (11.1% each). None of the patients were positive for Herpes simplex virus 1 or 2, Cytomegalovirus, Nipah or Chandipura viruses. Screening for bacterial aetiologies was negative for all patients. There were no distinguishable clinical or routine laboratory features between the different viral aetiologies.

**Conclusion:** A viral aetiology was identified in only about a quarter of patients with encephalitis. Dengue virus accounted for the majority. HSV accounted for none. This is the first identification of human WNV in Sri Lanka.
Clinical, laboratory profile and outcome of patients with dengue viral infection at a South Indian tertiary care hospital

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Background: Dengue is the most rapidly spreading mosquito-borne viral disease in the world with an estimated 50 million dengue infections occur annually. India is one of the seven identified countries in the South-East Asia region regularly reporting incidence of DF/DHF outbreaks and becoming major niche for dengue infection.

Methods & Materials: History, clinical examination findings, laboratory data, treatment details and outcome of patients with dengue fever was collected prospectively. SD BIO LINE DENGUE DUO (STANDARD DIAGNOSTICS, KOREA) kits were utilized to diagnose the dengue infection. This is a rapid diagnostic test which can detect simultaneously NS1 and antibodies of either Types (Ig M and Ig G). Patients were categorized into Undifferentiated DF, Dengue with Warning Signs and Severe Dengue according to WHO newer grading.

Results: Total 148 patients were included in the study. Among them 85(57 %) were male. There were 15(10%) in Undifferentiated DF, 45(31%) in DF with warning signs, 88(59%) in severe Dengue respectively. Mean age of our study population was 32.75±14.99, 33.43±14.95 respectively. Mean hospital stay in study population was 6.34±4.66 days. Rash was found in 47 (37.76%), hepatomegaly in 23 (15%) and splenomegaly seen in 25(16%) patients. Bleeding manifestations were seen 61(41%) patients in total study group, out of which malena was seen in 33(22%)patients, gum bleeding, hematuria, hematemesis, hemoptysis and epistaxis seen in 6(4%), 7(5%), 4(3%), 3(2%) and 3(2%) patients respectively. Intracranial bleed as bleeding manifestation was seen 2(1%) patients one SDH and one case of intracerebral bleed, both non traumatic. Mean hemoglobin (g/dl) for was 13.5±2.6g/dl. Hypoalbuminemia (<3.5 g/dl) was seen in 62(42%) patients with the mean value of 2.8± g/dl. Chest roentgenographic abnormalities found in 38(26%) patients of the study population. Pleural effusion was found in 31(19%) patients. Acalculous cholecystitis on ultrasonogram was seen in 18(12%) patients in our study population. NS1 antigen was detected in 80 (54%). Mortality was noted in 3.3% of the study population.
Conclusion: Out of 148 patients, majority consisted of severe dengue infection. Elevated transaminases were seen in 136 (92%) patients. Mortality was noted in 5(3.3)% of the study population.
Multiple siRNAs against HCV and host genes are more effective in inhibition of HCV replication

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Background: Hepatitis C virus is a major cause of chronic liver diseases such as chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. The present available anti-HCV drugs, Pegylated interferon-α (IFN-α) and Ribavirin have limited efficacy, resistance problems and high manufacturing cost. Among the newer approaches to curb the viral replication, RNA interference (RNAi) based approaches have shown tremendous prospective to inhibit HCV replication.

Methods & Materials: pFL-J6/JFH1 vector was linearized with XbaI and subjected to in vitro transcription. The HCV genomic replicon was transfected into Huh 7.5 cells for production of infectious HCV particles. The culture supernatant was collected to infect naive Huh 7.5 cells. siRNAs were targeted against NS5B region of HCV genome as well as cellular factors in order to down regulate HCV in cell model. Furthermore, multiple combinations of siRNAs were used to observe the additive HCV down regulation.

Results: Down regulation of La autoantigen, PSMA-7, hVAP-A and NS5B genes resulted in inhibition of HCV replication by about 65%, 30%, 35% and 40% respectively. Combination therapies of siRNAs against La autoantigen with NS5B and La autoantigen with hVAP-A resulted in ~ 85% inhibition in HCV replication.

Conclusion: Our findings indicate that in addition to HCV-specific siRNAs, siRNAs targeted to host cellular genes have showed promising down regulation in HCV replication. We also showed that simultaneous silencing of more than one target is more effective than silencing a single target to inhibit the viral replication. Therefore, multiple combinations of siRNAs against both the virus and host genes are likely to be a potent approach in the treatment of chronic hepatitis C.

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Background: Group A rotavirus induced acute gastroenteritis affects infants and children <5 years globally. Predominantly isolated rotavirus G types from human are G1, G2, G3 and G4 throughout the world including India. However, in recent years there has been an increased detection of G9 and G12 genotypes. Genotypes belonging to different animal origin such as G8, G10 and other rare genotypes have also been reported in humans with low frequency.

Methods & Materials: During an on-going hospital based surveillance study in 2014, stool samples were collected from patients (0-80 years) admitted with acute gastroenteritis at Infectious Disease (ID) Hospital, Kolkata. Presence of rotaviral VP6 protein was detected in stool samples using ELISA. The group A rotavirus positive samples were used for RNA extraction followed by Reverse Transcription and PCR. Cycle Sequencing and phylogenetic analysis were further used in the study.

Results: Sequence analysis of VP7 and VP4 gene segments of group A rotavirus positive samples identified two unusual genotypes namely G10P[14] and G12P[11]. The unusual nature of their G and P types led us to characterize the remaining nine gene segments for deciphering their evolutionary dynamics.

Conclusion: Our study reports the identification and characterization of unusual group A rotavirus strains i.e. G12P[11] and G10P[14] from Kolkata, India. The full genome sequencing highlighted interspecies transmission and multiple reassortment events in the origin of these strains. The G10P[14] strains possessed genomic constellation commonly found in artiodactyls and therefore probably have a zoonotic origin. The G12 strains predominantly carry Wa-like genomic backbone while P[11] type is derived from DS-1-like rotavirus strains and therefore possible reassortment events between the two genomic backbones might have led to the emergence of G12P[11] genotype. The study highlights the need for continued rotavirus surveillance and complete genetic characterization for monitoring unusual rotavirus strains and understanding their evolutionary origin.
Co-circulation of all four dengue virus serotypes with concurrent infections in a single dengue season
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Background: Dengue is one of the notable mosquito borne viral infections of public health concern. Dengue viruses (DENV) belongs to the genus Flavivirus and family Flaviviridae and has four antigenically related serotypes designated as DENV 1- 4. All the four serotypes can cause clinical manifestation ranging from mild self-limiting illness to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). However, severity in dengue viral infection is known to be affected by secondary infection with heterologous antibodies or with certain dengue virus serotypes and genotypes. This necessitates the study of circulating dengue serotypes in a particular locality. The present study reports for the first time the circulation of all the four dengue serotypes along with concurrent infections in an eastern state of India.

Methods & Materials: A total of 148 samples were received from clinically suspected dengue patients during September to December, 2014. All the 148 samples were subjected to dengue specific MAC ELISA (Pan Bio, Australia), and NS1 antigen detection by ELISA (Pan Bio, Australia) for detection of dengue IgM antibody and dengue NS1 antigen respectively. Twenty early acute samples (<3days of illness) received were subjected for detection of dengue viral RNA and serotyping using type specific nested multiplex RT-PCR.

Results: Twenty five samples were positive for dengue serology (dengue NS1 and/or dengue IgM Ab). Five samples were found to be positive for dengue viral RNA by RT-PCR. The type specific PCR revealed, Dengue type 2 (DENV-2) in 2 samples, DENV-4 was found in one and 2 samples were co-infected with DENV-1 and DENV-3. All the dengue positive patients had dengue fever and none had dengue hemorrhagic fever.

Conclusion: The present study reports for the first time the co-circulation of all the four dengue serotypes along with rarely detected DENV-4 for the first time from eastern India.
Elucidating the role of essential RNA secondary structural elements in dengue biology and their implication in dengue virulence

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**Background:** RNA-RNA interactions, central to many biological processes, are often mediated by various secondary structural elements of the RNA. In the context of single-stranded RNA viruses such as Dengue virus (DENV) and other flaviviruses, such RNA-RNA interactions may be the key to switching between translation and replication. DENV (serotypes 1-4) is the causative agent of Dengue fever (DF), Dengue hemorrhagic fever (DHF), and Dengue shock syndrome (DSS). Each of the DENV serotypes is further classified into several genotypes having varying degrees of pathogenicity and virulence. One of the conserved features of DENV and other flaviviruses is the presence of complementary sequences in the 5'- and 3'-untranslated regions (UTRs) that participate in long-range RNA-RNA interactions leading to the circularization of the genome. We hypothesized that the differences in secondary structures (and the corresponding three-dimensional orientation) of the 5' and 3' UTRs of the DENV RNA genome may underpin differences in virulence and pathogenicity of the different genotypes. Currently, there is no global scale analysis of DENV genomes correlating the RNA secondary structure with pathogenicity and virulence.

**Methods & Materials:** Towards this end, we have curated the NCBI database for full length genomes of DENV and classified them according to their respective genotypes. Using mFOLD, we derived the putative RNA secondary structures of the 5'- end of the RNA genome (encompassing the 5' UTR, the capsid hairpin (cHP), and the 5'- cyclization sequence (5' CS)) and the final 106 nucleotides of the 3'-UTR (comprising the 3'-SL and 3'-CS). Comparative analysis of the secondary structure elements of different genotypes was done using in-house software packages. We have also performed comparative analysis of these RNA structural elements across the serotypes.

**Results:** Our work has led to the observation of subtle but significant RNA secondary structure variations among not only the serotypes but within genotypes of a given serotype.

**Conclusion:** By carrying out an extensive global analysis of DENV genomic RNA secondary structure we were able to correlate serotype and genotype specific RNA secondary structural elements and their possible role in pathogenicity and virulence.
Hepatitis A virus outbreak in a compound in Tshwane district, Gauteng, South Africa: October 2014-March 2015

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**Background:** Hepatitis A is a contagious viral infection caused by hepatitis A virus (HAV). It is strongly associated with socio-economic indicators such as access to clean water and sanitation. On 16 February 2015, the Tshwane Outbreak Response Unit (ORU) was notified by a laboratory of an increase in blood samples that tested positive for HAV IgM. On the same day Tshwane ORU team was assembled to conduct further investigations.

**Methods & Materials:** We interviewed residents of a privately owned residential compound using a structured questionnaire collecting demographic, clinical and history of exposure information. A probable case was defined as a person who had an epidemiological link to a confirmed case with an onset of two or more HAV symptoms. A confirmed case was a probable case with laboratory confirmation of HAV. Blood samples were collected from the available residents for HAV IgM antibodies. We conducted an environmental assessment and noted access to water in food preparation areas, sanitary facilities, and the drainage system. Samples were collected from taps and water storage containers for chemical analysis.

**Results:** Of the 46 households with ~80 residents, 42 were interviewed. The median age was 24 years: range of 6-55 years. We collected 46 blood samples: 12 (26%) tested positive for HAV IgM. Among the 12 confirmed cases, HAV infection was equally distributed across both genders and 50% (n=6) were <15 years. There were three probable cases, including one food handler who prepared food in a communal kitchen along with four other women. There was no running water in the kitchen. Water access was restricted. Buckets were used for storage in the houses, flushing toilets and portable basins used to wash hands without soap. The drainage system was poor: sewage pipes leaked and toilets drained into an uncovered septic tank located in front of the kitchen. Water results showed that water was fit for human consumption.

**Conclusion:** Poor hand hygiene practices exacerbated by poor access to running water was the likely source of infection. Improving access to running water and sanitary facilities was recommended. Regular hand washing for all residents and improved environmental hygiene were emphasized.
Exploring the instability of reporters expressed under the subgenomic promoter in Chikungunya virus infectious cDNA clones

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Background: Reverse genetics employ the use of infectious cDNA clone to study the phenotype of a virus by introducing mutation in its genome. Previous studies using Alphavirus double subgenomic infectious cDNA clone encoding reporters, have met with the loss of reporter gene expression in subsequent passages. In the present work, we have tried to address the instability of such recombinant viruses of Chikungunya virus (CHIKV) with reporters upon passaging.

Methods & Materials: The infectious double subgenomic clone of CHIK encoding luciferase and EGFP was constructed under CMV promoter and the virus was rescued by direct DNA transfection in HEK293T cells. The virus was blindly passaged in Vero cells and monitored for EGFP expression and by reverse transcriptase PCR, using primers flanking the reporter gene. The presence of two variants of plasmid in the plasmid preparation and in the bacterial colony was also confirmed by PCR.

Results: We found that the recombinant virus upon passaging in Vero cells lost the EGFP expression (Fig 1a). The loss of EGFP was also confirmed from the viral RNA by reverse transcriptase PCR (Fig 1b). The plasmid clone preparation used to transfect HEK293T cells was examined for contamination by PCR that confirmed the presence of two variants of plasmids, with reporter and without reporter (Fig 2a). Re-transformation was done to remove contamination and to get a pure clone. The colony PCR on the transformants again confirmed the two variants of plasmids (Fig 2b). This could arise due to the recombination between two subgenomic promoters flanking the reporter, which causes accumulation of two variants of plasmids that in turn gives rise to two types of viruses. This in turn results in the efficient replication of the virus with a shorter genome than the recombinant virus with reporter and eventually the reporter is lost.

Conclusion: In conclusion we report the reason for the instability of the Alphavirus double subgenomic infectious cDNA clone, which arises due to recombination in the bacterial host and not in the mammalian cell. Plaque purification of the recombinant virus with reporter can be employed to recover the virus with reporter and express the foreign genes from the recombinant virus.
Inhibition of CCL2 dependent human cytomegalovirus replication by tricin
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Background: Human cytomegalovirus (HCMV) infection is a widespread opportunistic pathogen in immunocompromised individuals. So, HCMV infection is still associated with severe morbidity and mortality. HCMV can enhance the expression of a CC chemokines, CCL2 and CCL5. HCMV infection is presumed to contribute to atherosclerosis, where chemokines may have a pathogenic role. Elevated levels of CCL2 are observed in atherosclerotic plaques, where macrophages with the expression of a specific receptor for CCL2, CCR2, abundantly infiltrate. Thus, HCMV and CCL2 may cooperatively contribute to atherosclerosis. Our recent study revealed that tricin (4',5,7-trihydroxy-3',5'-dimethoxyflavone) has anti-HCMV activity in a human embryonic lung fibroblast cells (HEL). In the present study, we revealed that HCMV-induced CCL2 expression further augments HCMV infection and that tricin exerts its anti-HCMV activity by targeting CCL2.

Methods & Materials: HCMV Towne strain was propagated in HEL cells. Infectious virus production was titrated by using a plaque assay. The tricin compound used was synthetic. siRNAs targeting CCL2 and recombinant human CCL2 were purchased from a company. Proteins were detected using the ECL system by Western blot analysis. Gene expressions were detected using the reverse transcription quantitative real-time PCR analysis.

Results: We first examined the effects of HCMV infection on the expression of CCL2. HCMV infection induced CCL2 and CCR2 expression at the mRNA levels and the protein levels in HEL cells. CCL2 siRNA treatment reduced HCMV virion production, and this reduction was reversed by the addition of CCL2. We further observed that CCL2 siRNA, but not control siRNA, reduced the expression of HCMV immediate early gene and HCMV UL54 gene in a dose-dependent manner. Thus, HCMV infection can activate the CCL2-CCR2 interactions to further enhance HCMV infection and/or replication. Next, we observed that HCMV-induced CCL2 mRNA and protein expression was inhibited by tricin and exhibited inhibitory activities against HCMV replication. Thus, tricin exerts its anti-HCMV activities at least partly by inhibiting the expression of a CCL2, which can support HCMV infection and/or replication.

Conclusion: These results suggest that CCL2 is one of the chemokine involved in HCMV replication and tricin is a novel compound with potential anti-HCMV activity.
Epidemiology of dengue / dengue hemorrhagic fever in the northern Sri Lanka from 2009 to 2012

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Background: All 4 serotypes of dengue viruses (DENVs) have been co-circulating in Sri Lanka for more than 3 decades. However, people in the Northern part of Sri Lanka were isolated from the rest of the country due to travel restriction as a result of recently ended war. Thus the circulating DENV in the Northern part of Sri Lanka has not been investigated and this is such a study to describe the epidemiology of DENV serotypes and their association to DHF in the northern Sri Lanka from 2009 to 2012.

Methods & Materials: Demographic data and blood samples (5 mL / patient) were collected from 765 patients suspected of having DF / DHF from all medical and paediatric wards of the Teaching Hospital, Jaffna from 2009 to 2012. Viral RNA was extracted from patients’ sera using Qiagen viral RNA mini kit (Cat No 5206). Identification and typing of DENV were carried out using a combination of RT-PCR and a single-tube multiplex PCR. Primers described by Lanciotti et al were used to detect the C and PrM genes of the DENV.

Results: Of the 765 patients, 205 were positive for DENV RNA by the RT-PCR. Of the 205 RT-PCR positive patients, 64 were from 2009 / 2010 outbreak and the rest were from 2011/2012 outbreak. Distribution of DENV-1, DENV-2, DENV-3 and DENV-4 were found in 12 (18.7%), 19 (29.6%), 25 (39%) and 1 (1.5%) patients, respectively in the 2009/2010 outbreak. Seven (10.9%) had co-infection with DENV-2 and DENV-3. In contrast, in the 2011/2012 outbreak DENV-1 was found to be the dominant serotype (55.3%) and DENV-4 was not found in any patients. In 2009/2010 outbreak severe forms of DHF was caused by DENV-2 and DENV-3 (86%). However, in 2011/2012 more than one third cases of DHF were caused by DENV-1.

Conclusion: A shift in the circulating DENV serotypes was observed in the northern Sri Lanka. This shift might be due to the movement of people from the Northern Province Sri Lanka to other parts of the country and vice versa.
Interferon-gamma and IL-1beta activation precede death in neonatal mice models of central nervous system (CNS) infection by Chikungunya virus

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Background: The global outbreaks caused by the re-emerging strains of Chikungunya virus (CHIKV) have attained special significance with the reported incidences of CNS complications. The studies on the interferon and inflammatory cytokine responses show that they play a key role in the outcome of encephalitis caused by many alphaviruses. In order to understand the pathogenesis of CHIKV induced CNS infection, we explored the modulation of these cytokines in the brain tissue of neonatal mice models upon virus infection.

Methods & Materials: Balb/c mice, post-natal day 3 (PN3), (n= 6 each) were either infected subcutaneously with 10 plaque-forming units (pfu) of a low-passage clinical isolate of CHIKV or mock infected with the culture media alone. Mice were monitored daily for morbidity/ mortality and clinical manifestations. The brain from infected mice were collected after perfusing with ice cold PBS. Half the brain was used for transcript level analysis of immune genes by qRT-PCR while the other half was used for plaque assay to quantify the viral titre.

Results: PN3 Balb/c mice infected with 10 pfu of CHIKV developed the disease, with clinical symptoms progressing from day-3 post-infection (pi) and died on day 6-7 pi. The clinical manifestations started with lethargy (clinical score-1) on day 3; gradually progressed with alopecia and walking difficulties (score-2) on day 4; epileptic convulsions (score-3) on day 5; and death on day 6-7 (score- 4) (Fig-1). Peak viral load in brain was observed on day 6 by plaque assays, immediately prior to death (Fig-2). The mRNA levels of IFN-α and IFN-β were seen up regulated during the early days of infection, indicating a response to the ongoing viral replication in the brain tissues. TNF-α transcripts were up regulated at a moderate level at later days of infection. The most significant observation was the very high-level modulation of IL-1β, a potent pro-inflammatory cytokine, and IFN-γ, an immune cytokine activating robust T-cell response (Fig-3).

Conclusion: The cytokine transcripts IL-1β and IFN-γ, elucidated in response to the significant viral load in the brain, reached the peak level immediately prior to death in the animals indicating their key role in the fatal outcome of CNS infection with CHIKV.
Surveillance and molecular characterization of Rotavirus strains in hospitalized children with gastroenteritis in West Bengal

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Background: Rotavirus (RV) is a segmented, double-stranded RNA virus belonging to the Reoviridae family. It is a leading cause of severe gastroenteritis among children less than 5 years old. In India it was estimated 2 million visit outpatient and 457000–884000 hospitalized due to diarrhea disease. The aim of this study was to analyse prevalence and characterization of rotavirus strains among children admitted with gastroenteritis in two hospitals in West Bengal.

Methods & Materials: The rotavirus surveillance was carried at Medinipur Medical College & Hospital (MMCH), Medinipur and Institute of Child Health (ICH), Kolkata. The study was carried out between September 2013 to March 2015 and stool samples were collected from hospitalized children under 5yr of age admitted with gastroenteritis. The primary screening for rotavirus was carried out by ELISA, after conformation the G-P typing was done by multiplex semi-nested PCR and sequencing of VP7 and VP4 gene.

Results: During the surveillance period in ICH, 640 children admitted with diarrhoea and 601 samples were tested, of which 353 (58.73%) were rotavirus positive. In MMCH 775 children were admitted with diarrhoea and 755 samples were tested, of which 465 (61.6%) were rotavirus positive. In both hospitals, majority of children aged between 6 month to 24 month were found to be rotavirus positive. The most prevalent rotavirus strain detected from ICH was G1P[8] (54%) followed by G2P[4] (8.4%), G2P[6] (8.4%), G9P[4] (8.4%) and G9P[8] (4.2%), while at MMCH G1P[8] (75.9%) is most prevalent followed by G2P[4] (7%), G2P[6] (8.4%), G9P[4] (5%) and G9P[8] (5%), suggesting that in current surveillance period G1P[8] was most prevalent strains circulating in the Eastern India. In the phylogenetic dendrogram Eastern India strains cluster together forming a separate branch with respect to vaccine strain.

Conclusion: It was concluded that in both MMCH or ICH rotavirus accounts for >50% of admissions among children with diarrhea. The children aged between 6 month to 24 month are highly susceptible to rotavirus infection. In both regions the G1P[8] strains were highly prevalent during the surveillance period. The Eastern-India strains are highly similar to each other but show divergence to vaccine strains.
Prevalence of asymptomatic hepatitis B virus among sexually active youths in a rural community of Ebonyi state, Southeast Nigeria

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Background: Three major routes spread of hepatitis B virus in developing country such as Nigeria are prenatal, horizontal and sexual transmission. The main routes of transmission are neonatal with HBV carrier mother infecting her infant usually during birth or soon after birth following close contact, transfer of HBV via cuts, sexual transmission, transfusion of infected blood or blood products, needle stick injury, re-use of HBV contaminated needles, syringes, lancets and instruments including those used in tribal ceremonies.

Methods & Materials: This study was carried out to detect the prevalence of hepatitis B surface antigen (HBsAg) among healthy sexually active youths and adolescents in Ishiagu community, who voluntarily donated their blood for the study. In order to estimate the prevalence rate of HBsAg, 226 blood samples were screened by parallel diagnostic method using One Step Strip Style HBsAg test kits. The percentage prevalence of HBV infection was calculated by using patients with positive samples as numerator and the total numbers of the voluntary blood donor among the population of the study area as denominator. The data generated from this study were presented using descriptive statistics and chi-square to determine any significant relationship infection rate, age and gender.

Results: The overall prevalence rate of HBsAg of 8.9% was recorded. Age group (20-29) years had the highest prevalence of HBsAg (4%) compared to (3.1%) of the age group (10-19) years and (1.8%) of age group (30-39) years. HBsAg seropositivity was more prevalent among males (10.5%) than their female counterparts (9.26%). Age and Sex were statistically significant (p-value<0.05) by Chi-square test. This study confirmed that HBsAg is prevalent among screened asymptomatic healthy and sexually active youths in Ishiagu community.

Conclusion: Hence, general surveillance, mass immunization and public health education to stop the spread of the infection among general populace in Ishiagu community in Ivo Local Government of Ebonyi State is advocated. General surveillance through mass screening to identify those with infection and instituting appropriate treatments, mass immunization of the uninfected population against the virus and public health education to enlighten blood donors in entire Ishiagu Community of the possible risk factors and routes of infection are indeed advocated.
Molecular diversity of Hepatitis B virus (HBV) x gene: A preliminary report from Kerala
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Background: The HBV- x gene expresses a 154 aminoacid multifunctional protein widely associated with hepatocellular carcinoma in chronic patients. Genetic variability of the x gene may impact the differential oncogenic potential of HBV genotypes. This study attempts to investigate genetic variability of the HBV x gene in patients with chronic hepatitis B (CHBV) infection at a tertiary care hospital in Kerala, South India.

Methods & Materials: Blood samples from fifteen CHBV patients attending the gastroenterology unit of Pushpagiri Institute of Medical Science and Research Centre (PIMS & RC) were included. All samples were tested for HBsAg, HBeAg, HBc IgM, Anti HBs titre and biochemical tests {Aspartate transaminase (AST) & Alanine transaminase (ALT)}. All samples were screened by nested PCR, using primers targeting the core gene of HBV genome. HBV x gene was amplified using previously published primers and analysed by sequencing. All sequencing data were assembled, aligned and compared to the consensus HBV sequence to detect the mutations. Phylogenetic and mutation analyses were done using MEGA version 6.0.

Results: Of the 15 samples that were positive for HBV core gene, the HBV x gene was detected in 11 (73%) samples while four were negative. Majority of patients were infected with genotypes A (6, 54%) followed by D (5, 45%). All genotypes A strains belonged to subgenotype A1. The A1762T/G1764A double mutation in the BCP region, significantly related to HCC in earlier reported studies was found in 4 patients. Patients with genotype A1 had 50% risk of harbouring the 1762/1764 double mutation. The mutation C1485T in the x gene region were found in 10 samples. The mutation G1467A were observed in all patients with genotype A1.

Conclusion: A1 was the predominant subgenotype seen in this study. Mutations were significantly high in A1 strains. Association of these mutations with HCC/cirrhosis is unknown. Larger studies are essential for better understanding of the role of these mutations.
Hepatitis B virus genotypes and unique recombinants circulating among outpatients in selected hospitals in Kenya

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Background: HBV causes 780,000 deaths yearly worldwide. It’s endemic in Africa and of the ten known genotypes (A-J), A, D and E are prominent in Africa. Kenya is geographically located at the junction of these three genotypes and there is a paucity of HBV genotyping data. This study investigated molecular characterization of HBV circulating among outpatients of selected hospitals in Kenya.

Methods & Materials: 332 serum samples were obtained from patients with jaundice seeking medical services in four selected hospitals in Kenya. Hepatitis B surface Antigen and Antibody to the core antigen (HBsAg and HBcAb) were tested using commercial EIA (Elecsys, Roche Diagnostics). The HBsAg coding region was amplified and sequenced in all HBV DNA positive samples, with 20 specimens chosen at random for full genome sequence analysis.

Results: HBsAg positivity was 50.6% (168/332) with 66.9% showing DNA positivity among samples having sufficient volume for DNA testing (93/139). 2.0% were anti-HBc IgM positive, indicating acute infection. Based on HBsAg region sequencing, genotype A was predominant (90.3%), followed by genotype D (9.7%). HBV/D-infected individuals had a mean age of 43.0±2.0 years whereas HBV/A-infected individuals had a mean age of 34.0±4.0 years (Fisher test, p=0.02). Nucleotide distance measurements of the 20 full genome HBV/D sequences demonstrated two isolates having a distance >4% from all HBV/D subgenotypes and recombinants, suggesting a new HBV/D subgenotype. "Putative recombination between genotypes A and E was observed in a single isolate from the coastal region (Figure 3a), while putative genotype D/E recombinants were observed in 2 isolates from the western region (Figure 3b)."

Conclusion: Despite the availability of a stable HBV vaccine, the Prevalence of the virus was high among jaundice patients seeking health care. Genotype A1 is the predominant genotype in Kenya and in the Western region there are D/E recombinants. Finding of the novel HBV recombinant strains in Kenya indicates that further investigation is required as the morbidity and progression of infection with such strains is not known.
Molecular detection of enteroviruses in pigs in Lagos, southwestern Nigeria

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**Background:** Enteroviruses infect numerous mammalian species including humans, Non-Human Primates (NHP), sheeps, cows and pigs. Infections in pigs are mostly asymptomatic but can sometimes be severe and occasionally fatal. Enteroviruses in pigs are associated with a wide array of clinical presentations such as reproductive failure, diarrhoea and neurological disorders.

**Methods & Materials:** This study detected the enterovirus RNA from pigs in Ikeja Local Government Area of Lagos State, South-western Nigeria from a total of 110 (60 nasal and 50 faecal) samples from apparently healthy pigs in a major piggery between February and March, 2015 using reverse transcriptase polymerase chain reaction (RT-PCR). Amplification and detection were carried out to obtain enterovirus RNA specific bands of 154bp after agarose gel electrophoresis.

**Results:** Only 1 (0.9%) Enterovirus RNA positivity for specie and genus specific VP1 gene was detected from this study. Although the prevalence of Enteroviruses in pigs from this study is relatively low.

**Conclusion:** This still indicates a potential source of transmission to pig handlers, consumers and the general public at large. However, the continued surveillance of Enterovirus with the possibility of a wider coverage of the different regions within the state and the country at large is solicited.
Diarrhea in adult patients with influenza B

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**Background:** During 2014-2015 season we observed 147 cases of influenza. 34% cases were patients with influenza B, 49% - patients with several strains of influenza A/H3N2 and 17% - with influenza A/H1N1pdm2009.

**Methods & Materials:** Diagnosis was confirmed by PCR in nasal swabs. All patients received treatment in the central municipal hospital of Rostov-on-Don. Diarrhea was not recorded in patients with influenza A. 24% of patients with influenza B (12 cases) demonstrated diarrhea in the early stage of disease.

**Results:** All patients were women in the 50- to 65-year-old age group with the exception of the 26 year old pregnant woman who was an intravenous drug user. In all cases, patients had no epidemiological link and were hospitalized in a different period of time. In all cases the disease started with a fever up to 38.5 – 39 degrees C, weakness, headache. After a few hours of the onset of symptoms, 1-3 times vomiting developed in 42% of cases. Also, liquid stool without pathological admixtures 4-7 times a day throughout 2-3 days was recorded in 100% of patients since the first day of disease.

Before hospitalization 83% patients were treated with antipyretics. Patients admitted to the hospital on 1-2 day of disease with the preliminary diagnosis of acute infectious diarrhea.

Within 1-2 days of treatment in hospital dyspeptic symptoms disappeared and catarrhal symptoms started to dominate in the clinical picture of the disease: nasal congestion, sore throat, dry and wet cough. All patients with diarrhea had level of WBC count between 3.0-3.5 × 10⁹/L observed on the 2-3 day and 7-8 day of disease, radiological signs of acute bronchitis or bronchopneumonia were detected in 92% cases. Bacteriological investigation of feces did not detect any pathogenic enterobacteria, rotavirus and norovirus rapid tests and PCR on enterovirus were also negative. Mild or moderate dysbiosis of intestinal flora was detected in 100% cases. All patients were treated with crystalloids, sorbents, croseltamivir and antibiotics.

**Conclusion:** We assume that diarrhea in patients with influenza B in majority of cases associated with the development or worsening of common immunosuppression and dysbiotic changes in the intestine. It is often associated with bacterial complications.
Thrombocytopenia and anti-platelet antibodies in patients with chronic hepatitis C
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Background: One of the key position in hemostasis belongs to surface platelet receptors (SPR) - glycoproteins (GP) Ia/IIa, Ib/IX and IIb/IIIa. Antibodies to SPR can lead to development of thrombocytopenia in patients with chronic HCV-infection. However the role of different types of antibodies in this process is unknown.

Aim: to estimate the role of different types of antibodies to surface platelet receptors in HCV-associated thrombocytopenia.

Methods & Materials: the detection GP Ia/IIa, Ib/IX and IIb/IIIa was made by the ELISA technique in patients with HCV and thrombocytopenia (group 1, n=29), HCV without thrombocytopenia (group 2, n=28) and healthy people (group 3, n=32). All patients were untreated with interferon and ribavirin.

Results: Anti-GP Ia/IIa were detected in 44,8±9,2 % in 1th group, 3,6 ± 3,5% in 2th group, 12,5±5,8% in 3th group, p1-2<0,01, p1-3<0,05. Anti-GP Ib/IX were indicated in 55,2±9,2% in 1th group, 10,7±5,8% in 2th group, and 28,1±7,9% in 3th group, p1-2<0,01, p1-3<0,05. Anti- GP IIb/IIIa were recorded in the same groups in 24,1±8,0%, 17,8±7,3% and 25±7,6% accordingly without any statistical difference (Fig.1).

Mean optical density (MOD) of anti-GPIa/IIa in 1th group also was more significant than in 2th and 3th group (0,331±0,035, 0,208±0,015 and 0,217±0,012 accordingly, p1-2<0,01, p1-3<0,01).

More high level of anti-GPib/IIX MOD was also recorded in 1th group than in 2th group (0,280±0,025 and 0,215±0,010 p<0,05), no statistical evidence was found between 3th group and patients in 1th and 2th groups. Investigation of anti-GP IIb/IIIa MOD also did not find any statistical difference between patients with HCV-infected patients (both groups) and healthy people (Fig.2)

Conclusion: High frequency of revealing and significant level of MOD of anti-GP Ia/IIa и Ib/IX in HCV-infected patients with thrombocytopenia demonstrated the evidence of damage in the first stages of primary hemostasis: adhesion of platelets to collagen with support of GP Ia/IIa and stabilization of this aggregate with support of collagen-binding activity of GP Ib/IX (factor Von Willebrand) as well as more high risk of destruction platelets binding with anti-GP Ia/IIa and anti-GP Ib/IX in spleen. Detection of anti-GP Ia/IIa и Ib/IX can serve as prognostication criteria for developing thrombocytopenia in patients with chronic HCV-infection.
Indian experience with use of sofosbuvir for treatment of hepatitis C virus infection: Preliminary data from southern India

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**Background:** Hepatitis C virus (HCV) infection is a major health problem worldwide. India has a burden of over 10 million HCV chronic carriers. The previous standard of care for HCV was pegylated IFN (Peg IFN) and ribavirin (RBV). The new direct acting antivirals that are now available promise sustained virological response, tolerability and higher cure rates. This study aims to look at the response to sofosbuvir (SOF) (an NS5B nucleotide analog) in combination with RBV with/ without Peg IFN among Indian patients.

**Methods & Materials:** Plasma from 44 patients (including 5 post transplant) was obtained from those who were treated with SOF and RBV ± PegIFN. RNA extraction and amplification was done using real time PCR. HCV genotypes were ascertained using the NS5B region based sequencing. Samples were tested for RNA negativity, one month after treatment initiation (RVR), and 3 to 6 months later.

**Results:** Thirty eight of 44 patients were genotyped. Genotype distribution was 7(18.4%) for type 1, 28(73.7%) for type3, 3(9%) for type 4 respectively. Patients with 1, 3 & 4 genotypes on additional Peg IFN was 4(10.5%), 5(14.7%) & 1(2.94%) and without Peg IFN was 3(7.9%), 23(60.5%) & 2(5.9%). RVR for all patients was 100% and at ≥3 month follow up, there was 100% response to SOF based therapy. RVR for the 5 post transplant patients was 100%.

**Conclusion:** This preliminary study in this tertiary care centre shows a 100% rapid virus response to SOF based therapy irrespective of genotype and transplant status among Indian patients. Long term prospective studies are warranted to estimate sustained virological response to Sofosbuvir in Indian patients
Serum levels of soluble CD26, a novel prognostic marker for acute hepatitis E infection

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**Background:** Even without treatment, most of acute hepatitis E virus (HEV) infected patients resolve HEV but sometimes the disease leads to acute liver failure, chronic infection, or extrahepatic symptoms. The mechanisms of HEV pathogenesis appear to be substantially immune mediated. However, the immune responses to HEV are not precisely identified. The aim of this study was evaluation of Th1/Th2 ratio by determining serum soluble markers from Th1 and Th2 cells in acute HEV infected patients.

**Methods & Materials:** This case control study included 35 acute HEV infected patients and 35 age and sex matched anti-HEV negative healthy controls. The serum levels of IFN-g, IL-4, soluble CD26 (sCD26) and sCD30 were determined by enzyme-linked immunosorbent assay.

**Results:** The results showed a significant difference in IFN-g and sCD26 ($P<0.0001$ and $P=0.001$) but not IL-4 and sCD30 ($P=0.354$ and $P=0.159$) between acute HEV patients and controls, respectively. There was only a positive direct correlation between serum levels of sCD26 and IFN-g in acute HEV patients ($r=0.64$, $P=0.001$). In addition, the ratio of sCD26/sCD30 in acute HEV group was more than two fold higher than in HEV negative controls.

**Conclusion:** Acute HEV infection shows a pattern of Th1-type immune response and a direct significant positive correlation between the serum level of sCD26 and IFN-g in acute HEV infected patients, suggesting that the trend of sCD26 levels is a valuable marker for predicting hepatic inflammation in hepatitis E.
Splenic infarction, a rare complication of infectious mononucleosis in a patient with no significant comorbidity: Case report and review of the literature

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Background: We report a case of a 32-y-old male diagnosed with infectious mononucleosis with splenomegaly complicated by splenic infarct in second week of illness documented by abdominal CT scan.

Methods & Materials: Infectious mononucleosis is commonly caused by an Epstein – Barr virus (EBV) infection. The clinical syndrome usually presents with the classic triad of pharyngitis, fever, and lymphadenopathy. Hepatosplenomegaly is also common. [1]. Splenic infarction represents a very rare complication of infectious mononucleosis, encountered mostly in patients with underlying comorbidities.

Results: A 32yr old male was admitted to our department for high-grade fever and upper respiratory tract infection for 10 days. Cervical lymphadenopathy and hepatosplenomegaly was also found during the physical examination and on abdominal ultrasound. Routine laboratory results showed a white blood cell count of 5K with 67% lymphocytes, including 10% of atypical lymphocytes, and elevated levels of aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase. Serologic viral screening for hepatitis B and C viruses, human immunodeficiency virus, cytomegalovirus was negative. EBV was detected, EBV viral capsid antigen immunoglobulin M (VCA-IgM) antibodies at high titer. A monospot test was also positive.

On tenth day of illness patient complained of acute left upper quadrant pain. Ultrasound examination revealed a hypoechoicogenic area (5.1cm-3.2cm) in the enlarged spleen. Abdominal CT scan was carried out and showed a splenic infarct (size 9.6 _ 6.4 cm) (Figure 1). Patient had negative Coombs’ tests, negative thrombophilic screen (activated protein C resistance, protein C and S levels, lupus anticoagulant, anticardiolipin, and anti- â 2 glycoprotein I antibodies)

Echocardiography, vasculitic profile ANA, C- ANCA , P-ANCA and bone marrow aspiration and biopsy were unremarkable.

The patient was managed symptomatically and showed resolution of infarct at 8 weeks.

Conclusion: Splenic infarction represents a very rare complication of infectious mononucleosis due to EBV infection. mostly seen due to sickle-cell disease [2], pyruvate kinase deficiency [3] autoimmune haemolytic anaemia[4], hereditary spherocytosis [5], and protein C deficiency [6]. Acute EBV infection has recently been recognized as a potential cause of splenic infarction, with or without comorbidities studied in the largest series of patients with splenic infarction [7].
Cytokine profile in response to Chikungunya virus (CHIKV) associated with CHIKV polyarthritis in acute febrile patients from South India

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Background: Chikungunya causes an acute febrile illness with painful syndrome, asthenia, skin rash, and polyarthritis. In some cases polyarthralgia can last for months to year. Understanding cytokine profile in CHIKV may be helpful to determine severity of the disease.

Methods & Materials: Acute phase blood samples were collected from CHIKV suspected cases during an outbreak in northern districts of Kerala in 2008 and 2009. Samples were processed and stored at -80°C. All the collected samples were screened by CHIKV RT-PCR targeting E2 region (305bp). Among 237 samples collected from an outbreak, 51 chikv suspected samples (20 PCR positives and 10 PCR negatives) and 10 healthy controls were tested with cytokine ELISA. IL-6, IL-8, IL-10, TNF-α, and IFN-γ single analyte cytokine quantification ELISA were performed.

Results: Among 237 collected during the outbreak, 51 (21.5%) samples were confirmed as CHIKV by RT-PCR. Majority the patients with severe joint pain was much higher in 2009 (85%) than in 2008 (26%). Joint pain was found more among chikv PCR positive patients when compare to CHIKV PCR negatives (p = 0.04) and it was statistically significance. Cytokines detection ranged from 13.28-72.32pg/ml, mean- 16.4 for IL-6, 7.65-322.01pg/ml, mean-84.5 for IL-8, 49.95–166.71pg/ml, mean-78.3 for IFN-γ. IL-8, IL-6 and IFN-γ were found to be elevated among chikv patients when compare to control (p=0.00001, 0.004 and 0.005) respectively and it was statistically significance. TNF-α and IL-10 were not significantly increased (p=0.09, p=0.16). Majority of the patients (96%) reported with joint pain shown increased level of IL-8. New vector adaptability of CHIKV may cause for the severity of joint pain during outbreak.

Conclusion: Determination of specific immune mediators involved in the disease progression may be helpful for therapeutic interventions. IL-8 may be used as early biomarker for CHIKV infection to understand severity of the disease.
Elucidation of viral load and host immune responses as severity predictors of acute lower respiratory tract infections (ALRTI) mediated by respiratory syncytial virus (RSV) and human metapneumovirus (hMPV)

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Background: RSV and hMPV are the most significant causes of severe lower respiratory tract infections with high mortality in children under 2 years of age worldwide. Disease pathogenesis is yet to be ascertained however host immune mechanisms and viral load might play some role. The present study is undertaken to explore the correlating factors in terms of RSV & hMPV load and host immune responses to predict the ALRTI severity.

Methods & Materials: NPAs were collected from children (n=142) admitted in PGIMER, Chandigarh with ALRTI from December 2014 -October 2015. Samples positive for RSV and hMPV by RT PCR targeting N gene were subjected for viral load estimation by Real Time PCR using CDC recommended primer-probes. The level of IL-17A, IFN-Ɣ, TNF-α, IL-10, IL-6, IL-4 & IL-2 in NPA samples were determined in flow cytometry by cytokine bead array. The viral load and cytokine levels were correlated with the WHO guidelines of ALRTI severity determination. The statistical significance was calculated with SPSS 16.0.

Results: Of the 142 patients, 74(52.1%) presented with bronchiolitis, 63(44.5%)with pneumonia and 5(3.3%)with Wheezing. 51 patients (35.91%) were classified under ALRTI, 63 (44.36%) severe ALRTI and 28 (19.71%) very severe ALRTI. RSV and hMPV positivity were seen in 56 (39.42%) and 14 (9.85%) patients respectively. Bronchiolitis was the most common clinical presentation (55.4%) in RSV infected children. RSV viral load amounting to 2.75X10⁵, 1.22X10⁵ and 1.16X10⁵/µl of 500ng RNA were seen in ALRTI, severe ALRTI and very severe ALRTI patients respectively (p=0.613). The mean value of IL-17A in RSV infected very severe ALRTI patients was 144.38pg/ml followed be 115.65pg/ml in severe ALRTI and 80.40 pg/ml in ALRTI groups. IL-10 level in very severe ALRTI with RSV was 714.1 pg/ml whereas 132.90 pg/ml in severe ALRTI patients. The mean value of cytokines in hMPV patients were found to be as 171.43pg/ml (IL-6), 334.82 pg/ml (IL-10), 23.14pg/ml (TNF-α), 100.46pg/ml (IFN-Ɣ) and 86.33 pg/ml (IL-17).

Conclusion: Future study involving large group of patients to elucidate viral factors and immune markers as a predictor of severity will be extremely helpful for ALRTI management and disease intervention.
Genetic variability and molecular evolution of hepatitis B virus in HIV co-infected patients on lamivudine based anti-retroviral therapy: A 5 year longitudinal study
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Background: Reports on the concomitant impact of HIV infection and long term cross-reactive HAART on the genetic variability and molecular evolution of HBV over time are limited. This longitudinal study retrospectively investigated the molecular characteristics of chronic HBV in HIV co-infected patients on lamivudine (3TC)-based HAART, over the course of a 5 year period.

Methods & Materials: Four HIV co-infected patients consecutively recruited and followed-up from 2004 to 2008 were screened for complete hepatitis B seromarkers and viral loads determined. HBV full length genome isolates were amplified and directly sequenced from serial samples. Evolutionary analyses were then conducted and prediction of phenotypic drug resistance and escape mutations carried out.

Results: All patients exhibited persistent chronic HBV infection at baseline, prior to 3TC-based HAART initiation and over the course of follow-up. Based on phylogenetic clustering, it could be determined that all but one patient were infected with HBV subgenotype A1. The greatest evolutionary distance determined was 0.008 (~25 base substitutions within the 3.2 Kb genome) after 5 years of persistent chronic HBV infection with exposure to 3TC-based HAART. Positive selection pressure, based on $dS/dN$ ratios <0.05, was evident within structural genes (pre-S/S and Pre-C/C). It was noted that the pol ORF in all study patients' isolates was relatively variable prior to HAART initiation at baseline and during the course of follow-up.

Conclusion: Overall, HBV exhibited limited evolutionary rates even after 5 years, which could be attributed to the minimal genetic diversity observed. The impact of current TDF-based HAART regimens on the molecular evolution of HBV in HIV co-infected South African patients should be investigated.
Elevation in liver enzymes are associated with increased IL-2 and may predict severe outcomes of dengue virus infection in a Sri Lankan cohort

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Background: A synergistic effect of host genetic factors, host immunity and the virulence of dengue viruses (DENV) contribute to the pathogenesis of DENV infections. In severe DENV infections, the hepatic transaminase AST level increases more than the hepatic transaminase ALT. IL-2 and TNF-α are both elevated in DENV infection as a part of the body’s early response to infection. The objective was to assess the correlation between changes in IL-2 and TNF-α levels with changes in liver enzymes in dengue patients with varying clinical severity.

Methods & Materials: A total of 67 DENV infected patients (DF=24 and DHF=43) either confirmed by ELISA or RT-PCR from July 2011 to February 2012 from General Hospital Kandy were selected for the IL-2 and TNF-α evaluation using a single analyte ELISArray (Qiagen, Germany). Clinical, haematological parameters and hepatic transaminases (AST and ALT) were recorded on admission. Five mL of blood was collected from DENV suspected patients on fever days 5 or less (onset of fever was considered as day 1).

Results: Of the patients, 47.76% (n=32) showed AST: ALT >2. AST: ALT mean ±SD among DF was 1.64±0.74 U/L while it was 3.18± 4.50 U/L for DHF/DSS patients. No significant correlation was noted between AST: ALT and TNF-α and also with IL-2. A significant positive linear correlation was observed between AST and IL-2 levels (r= 0.31 p = 0.01) and also between ALT and IL-2 levels (r= 0.27 p = 0.02). No significant correlation was noted between AST and TNF-α and ALT and IL-2.

Conclusion: Almost half of our study population showed AST: ALT>2 indicating acute changes in liver function and the potential for liver derangement due to DENV infection. There was a statistically significant positive correlation between the IL-2 with AST and ALT levels, although no correlation was noted between AST and ALT with TNF-α. The positive correlations between elevations of AST and ALT with IL-2, and the association of higher levels of these factors in DHF/DSS compared with DF suggest that these measurements may be useful predictors for the progression of DENV infection to severe DF/DHF.
Co-infections with multiple dengue virus serotypes in patients from 3 different Provinces of Sri Lanka, a dengue hyper endemic country

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Background: The circulation of multiple dengue viral (DENV) serotypes in a same locale has caused people to get infected with mixed DENV serotypes in subsequent or simultaneous infections. The objective was to study the clinical presentations together with reverse transcriptase PCR (RT-PCR) and serology of co-infections to identify pattern of disease severity among co-infections in patients from 3 different provinces of Sri Lanka.

Methods & Materials: Clinically diagnosed dengue fever (DF) / dengue haemorrhagic fever (DHF) patients from Teaching Hospitals, Jaffna and Kandy and General Hospitals, Gampaha and Negambo with fever days less than 5 were included. Clinical and haematological data were assessed. DENV capsid gene detection was performed by RT-PCR followed by DENV sero-typing. DENV IgM/IgG detection were performed using ELISA.

Results: Out of the 1249 RT-PCR performed on patients during 2009-2012, 329 were RT-PCR positive and of which 34/329 (10.33%) patients had DENV co-infections with two or more serotypes. In these three Provinces all 4 DENV serotypes were found to be co-circulating during 2009-2012 and DENV-1 was the predominant serotype circulated in all 3 provinces. Highest number of co-infection (17/34) was DENV-1 with DENV-2. Of 34 co-infected patients, 24 were diagnosed as DF and the rest were DHF (n=10). There were 16 primary and 18 secondary DENV infections. Out of the primary DENV infections 12/16 were DF and the rest 4/16 were DHF. In the secondary DENV infections 22/28 were DF and 6/28 were DHF. No significant difference was noted between the total white blood cell count and platelet counts in monotypic and co-infections with multiple DENV serotypes.

Conclusion: In this population DENV-1 was the dominant DENV serotype followed by DENV-2. Presence of DENV co-infections in all 3 provinces indicates the hyperendemicity of DENV throughout the country. The absence of significant association of disease severity between the monotypic and co-infections with multiple DENV serotypes point out the progression of the disease into severe forms driven by factors other than viral factors. The presence of DENV co-infections may also lead to recombination of genetic components contributing to the emergence of new DENV strains that might be more virulent and aggressive in causing severe dengue.
Upsurge in vaccine preventable hepatitis A virus infection in adult patients from a tertiary care hospital of North India

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Background: Prevalence of acute viral hepatitis A among adult in developing country is low due to pre exposure of Hepatitis A virus (HAV) during childhood and adolescence. An increase in acute viral hepatitis A infection among admitted adult patients is being observed in this centre. Hence, study done to know the prevalence of Hepatitis A virus (HAV) and Hepatitis E virus (HEV) in adult patients of acute hepatitis admitted in Gastroenterology Department, Nehru Hospital, PGIMER, Chandigarh.

Methods & Materials: Two hundred eighty five adult patients (206 Males, 79 Females) of acute hepatitis, alcoholic liver disease with acute exacerbations and chronic liver disease with decompensation were included in the study for suspected viral aetiology (Study Period: October 2012 to September 2015). Patient's detail record included clinical features, routine diagnostic investigations and abdominal ultra-sonography. Three ml. of blood was collected from each patient and serum stored at –20°C. All samples were tested uniformly for Anti HAV IgM, Anti HEV IgM, HBsAg and Anti HCV by ELISA.

Results: Overall 89 adult patients (31.22%, 67 Males, 22 Females, Mean age: 41.31 yrs. + 14.74) out of 285 patients of suspected viral hepatitis were reactive for Anti HAV IgM. 4 patients (1.4%, all male) were reactive only for anti HEV IgM. 5 patients (1.75%, 4 male, 1 female) were HBsAg reactive while 4 patients (1.4%, 3 male, 1 female) were anti HCV reactive. Dual viral infections were found in 7 patients (2.45%, 6 male, 1 female) which were reactive for Anti HAV IgM + HBsAg, 8 patients (2.8%, 6 male, 2 female) were reactive for Anti HAV IgM + Anti HCV, and 01 male patient was reactive for Anti HAV IgM + Anti HEV IgM.

Conclusion: 31.22% adult patients of suspected viral hepatitis had acute viral hepatitis A infection needing hospitalization. 2.8% of adult patients had dual HAV and HCV infection while 2.45% adults had dual HAV and HBV infection. Prophylactic vaccination against HAV infection is needed for adult patients including healthy persons from this region after appropriate screening for HAV immunity.
Prevalence of hepatitis C virus infection among asymptomatic Pakistani children

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\textbf{Background:} In the current era, viral hepatic infection HCV has become widespread and is the most important reason of liver disease, worldwide. This study was conducted to determine the prevalence of hepatitis C virus (HCV) infection in patients admitted in children ward and attending children outdoor, at Akhtar Saeed hospital, Lahore (a teaching trust hospital).

\textbf{Methods & Materials:} In this cross-sectional descriptive study, 1358 asymptomatic patients attending department of Pediatrics were selected randomly. This study was conducted from March 2014 to March 2015. Patients of either sex, were included. The ratio of male to female was 50:50. The age ranged from 6 months to two years. Screening for antibodies against HCV (anti-HCV) was performed through Kit method and positive cases were confirmed by ELISA. Informed verbal consent was taken. Data was analyzed by using SPSS 16.0.

\textbf{Results:} Out of 1358 registered patients, 4 patients were found reactive and confirmed on ELISA. The overall sero-prevalence of HCV infection within the study period was 0.33%. This study was conducted from March 2014 to March 2015. Patients of either sex, were included. The ratio of male to female was 50:50. The age ranged from 6 months to two years. Screening for antibodies against HCV (anti-HCV) was performed through Kit method and positive cases were confirmed by ELISA. Informed verbal consent was taken. Data was analyzed by using SPSS 16.0.

\textbf{Conclusion:} Data showed only 4 out of 1358 asymptomatic patients had Anti HCV positive. Undiagnosed, asymptomatic patients may be a basis of infectivity in many ways like by intimate individual contact with other family members. Evading unnecessary blood transfusion and injections and execution of strict infection control measures are highly recommended to trim down the frequency of HCV infection hospital, Lahore (a teaching trust hospital).
Delayed appearance of virus induced morphological changes in cultures derived from dengue and dengue haemorrhagic fever patients

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**Background:** The replication of dengue virus (DENV) is still not fully understood. Virus induced cytopathic effect (CPE) observed under light microscopy during DENV replication include rapid destruction of infected cells, which begins with rounding of cells and syncytia formation. However, these cellular changes are more pronounced in in vitro infections with wild type DENV than the laboratory adapted DENV, suggesting an increased vigor and virulence of evolving wild type DENV. The objective of the present study was to evaluate the CPE in C6/36 cells infected with sera of dengue (DF) and dengue haemorrhagic fever (DHF) patients.

**Methods & Materials:** Fifty-three RT-PCR positive serum samples of fever day 3 - 5 collected from Gampaha and Negombo Hospitals were used for the study. C6/36 cells were infected with 100 µl of DF (n=37) and DHF (n=16) patients’ sera.

**Results:** Out of 53 sera, 24 were DENV-1; 17 were DENV-2; 1 was DENV-3 and 11 samples showed mixed infections. The percentages of infection in DF and DHF were calculated using a mathematical formula.

DF,
\[ y = -0.3367x^5 + 7.6333x^4 - 65.233x^3 + 256.47x^2 - 430.23x + 250.5 \]

DHF,
\[ y = -0.4062x^4 + 8.7431x^3 - 70.719x^2 + 263.17x - 300.54 \]

(y = % of infection in DF or DHF; x = day of CPE [2≤x≤7 days])

The actual time taken for the development of CPE in > 90% of the cells in DF sera infected cultures was 38.4 hours post-infection and in DHF sera infected cultures was 43.2 hours. The time taken for the development of CPE was affected by the source of the virus (DF or DHF) but not the DENV serotypes. When we infected the Vero cells using the same DF / DHF serum samples, the cultures took more than 15 days to develop CPE.

**Conclusion:** The difference in the time required to infect the cells by the DENV derived from DF and DHF patients might be related to different mechanisms of DENV replication or the virulence of the virus. The difference in the time required to infect the two cell lines may be useful in the study of host-pathogen interactions on virus entry, replication and exit.
Phylogenetic analysis of the complete genome of the APMV-13 isolate from Ukraine
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\textbf{Background:} Avian paramyxoviruses (APMVs) belong to the genus \textit{Avulavirus} of the family \textit{Paramyxoviridae} and have been shown to infect a wide variety of poultry and wild bird species. Up to date, the International Committee on Taxonomy of viruses have officially approved 9 serotypes of APMV (APMV 1 – 9), but recently four new serotypes have been described (APMV 10 – 13). A member of the putative new serotype APMV 13 was isolated from white-fronted goose on the territory of Askania-Nova National Park (Ukraine) in 2011.

\textbf{Methods & Materials:} The virus was propagated in specific-pathogen-free embryonated chicken eggs and preliminary analyses of the isolate were performed by Sanger sequencing. Based on the obtained results, primers were designed: APMV13-3000 CTGTCGCAgTAATGGAAgGAA/BIOTIN, APMV13-6000 GGGAAgGCCTCTCTTgATTGAT/BIOTIN and APMV13-12000 GCTgGGACTgCgCgCTATCAAT/BIOTIN/ and isolate was further processed by next-generation sequencing (NGS). The phylogenetic analysis was performed with the software MEGA6.06 and the evolutionary history was inferred by using 52 full genome sequencing data of APMVs and the Neighbor joining methods, bootstrap 1000 (Tamura, Stecher, Peterson, Filipski, and Kumar 2013).

\textbf{Results:} The complete genome contains six transcriptional units (3’-NP-P-M-F-HN-L-5’) of 1482, 1194, 1101, 1653, 1740 and 6639 nucleotides in length, respectively. Amino acid based phylogenetic analysis of coding regions revealed closest relationship to APMV 12, however low amino acid identity (52% to 75%) was observed. The fusion protein gene of the virus has 98% nucleotide identity to APMV/goose/Shimane/67/2000, isolated from a fecal sample collected from a goose and reported previously as APMV 13. Phylogenetic evaluation of coding regions revealed that predicted amino acid sequences of all other proteins were more closely related to APMV-12 (Np has 75.1 \% sequence identity, P-52.1 \%, M-73.1 \%, F-69.5 \%, HN-63.7 \%, L-66.0 \%) than to the rest of the serotypes.

\textbf{Conclusion:} Our data highlights the importance of continuous monitoring of wild birds in the Azov-Black Sea region and to further identify possible introductions of avian viruses from other geographic regions. This project was partially funded by Project Agreement P-568 funded by the Department of State, and by the Defense Threat Reduction Agency USDA/ARS #685/FRCALL 12-6-2-0005.
Characterisation of chronic hepatitis B virus carriers with viral load and correlation with other viral markers

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Background: According to WHO, India has around 40 million chronic HBV carriers. This study was carried out with objectives; profiling of viral markers in HBV carriers with viral load, correlation of ALT levels, HBe Ag status with their viral load, to determine the mutation and response to antiviral therapy in treated and treatment naïve individuals.

Methods & Materials: DNA was extracted from plasma by Qiagen DNA blood mini kit (Qiagen, Germany). HBV viral load was estimated by Artus HBV real time PCR kit (Qiagen, Germany) in ABI HT fast real time PCR platform (Applied Biosystems, USA). Randomly 27 high viral load samples were chosen and polymerase gene was amplified using specific primers by Platinum Taq DNA polymerase kit (Invitrogen, USA) and sanger sequencing (Big Dye terminator kit, ABI, USA) was performed to identify the drug resistance mutations and genotyping. Mutational analysis was done by HBV geno2pheno software.

Results: Among 1129 samples tested for viral loads, 26% (n=295) had high viral load (Median HBV viral load is 7×10^5 IU/ml, range: 2×10^3 IU/ml to 4×10^7 IU/ml), of which 113 samples had viral load between 2000 IU/ml - 20,000 IU/ml and 182 had more than 20,000 IU/ml. 31% had detectable viral load below 2000 IU/ml.. HBe Ag status were checked for these patients, HBe Ag status were known for 545 patients, 26% (n=141) were found positive for HBe Ag, 13.7% had high viral load with HBe Ag negative. HBe Ag positivity was positively correlated with the high viral load with p<0.001 (STATA II software). Elevated ALT levels were seen in patients with high viral load and the correlation was significant with p<0.001. Among 27 individuals, mutation rtM204V along with rtL180M was seen in one treated individual and a compensatory mutation was observed in other treatment naïve individual.

Conclusion: Periodic monitoring of these patients for factors like viral load, HBe Ag status and ALT level will enable the clinician to initiate appropriate therapy at the right time to obtain sustained virological response.
Changing trend of rotavirus strains circulating in children <5 years in Delhi

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Background: Rotaviruses evolve rapidly by reassorting their gene segments. This enhances the potentiality of high genomic diversity and fast adaptation to the changing environmental conditions. A huge diversity in rotavirus strains emphasizes the importance of continuous monitoring of strains circulating in the population. This study compares the incidence of rotavirus strains circulating in children <5 years in Delhi during 2013-2014 with 2009-2010.

Methods & Materials: Diarrheal children <5 years admitted to Kalawati Saran Children Hospital during 2013-2014 and 2009-2010 were included in the study. The stool specimens collected from children were examined for rotavirus antigen by enzyme immunoassay. Rotavirus positive specimens were G and P genotyped by multiplex seminested RT-PCR.

Results: Rotavirus antigen was found in 170/350 (48.6%) and 106/243 (43.6%) samples during the two study periods. G1P[8], G9P[4] and G12P[6] were common strains during the present study with 38.2%, 11.2% and 10% detection rates respectively. Whereas G9P[4] (13.2%), G1P[8] (11.32%) and G2P[6] (9.43%) were common earlier. G12P[6] was highly observed in the present study, instead G2P[6] was prevalent earlier. Amongst G genotypes there was a high dominance of G1 (58.2%), G9 (15.3%) and G12 (14.1%) genotypes in the present study compared to previous study where G9 (32%), G1 (27.3%) and G2 (19.81%) were predominant. G2 (2.3%) genotype was rarely observed in the present study, but was the third highest (19.8%) G genotype observed earlier. Of note was the complete disappearance of G3 genotype in both the study periods and G4 genotype in the present study. P[4], P[6] and P[8] were the common P genotypes in both the studies, but P[8] (43%) was more common in the present study and P [4] (25.5%) in previous. We found nearly equal percentages of G and P mixed infections in both the studies while G non typeables (6.6%) were more common in the previous study and P non typeables P (16.7%) in the present study.

Conclusion: The Study highlights the high disease burden of rotavirus infection in children <5 years. Rotavirus strains circulating in the population were found to change with time. The study therefore, emphasizes the need for continued and regular analysis of genotypic behavior of emerging strains.
Molecular detection of Chikungunya virus infection during 2013-2014 in Delhi

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Background: Chikungunya fever is an emerging arboviral disease that caused by the chikungunya virus (Chikv). While many infected individuals recover after primary illness, others suffer from persistent debilitating arthralgia that can last for months to years. Often Chikv infections go undiagnosed because the clinical symptoms mirror those of Dengue virus infections. There is need to have data on the prevalence of Chikv infections; we, therefore used molecular diagnostic test for diagnosis of Chikv infections over a 2-year period in a tertiary care hospital in New Delhi.

Methods & Materials: We enrolled patients suspected to have chikungunya infections with fever ≤ 7 days duration at the time of their visit to the Out/Inpatient department of AIIMS hospital. Blood samples were collected from 143 patients suspected to have chikungunya infection from Jan 2013 to Dec 2014. All samples were screened by RT-PCR (One step RT-PCR kit, QIAgen) and IgM ELISA (SD Chikungunya IgM ELISA kit). Statistical analysis was performed using STATA12.

Results: Of the 143 subjects, 34 (23.78%) were found positive by either RT-PCR or IgM ELISA; 20 (58.82 %) were males and 14 (41.17%) female; 18(53%) were adults. Mean (SD) age was 21.02 (16.40) in Chikv infected patients; this was similar to those without Chikv infections. Days of fever of Chikv confirmed cases at 4.7 (5.3) were comparable to 3.8 (2.3) of Chikv negative cases (P value: 0.13). Chikungunya infections appear to be frequently associated with headache, myalgia, rashes, joint pain and joint swelling. Most of the Chikv positive cases diagnosed in adult population rather than children.

Conclusion: Our results suggest a higher prevalence of Chikv infection with adults being more commonly affected. The study emphasizes the need for continuous surveillance for disease burden using multiple diagnostic tests and also warrants the need for an appropriate molecular diagnostic for early detection of Chikv infection.
Detection of rotavirus in diarrhoeic children from 0-5 years of age in Kano North-Western Nigeria

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Background: Rotavirus (RV) is the most common cause of severe diarrhoeal illness in infants and young children 0-5 years of age in both developing and developed countries. Rotavirus infection has been estimated to result in 453,000 deaths of young children in developed and developing parts of the world. However, limited data exist on rotavirus (RV) infection in Kano, North-Western Nigeria.

Methods & Materials: This Study was aimed at determining the prevalence and genotyping of rotavirus among these children using Enzyme Linked ImmunoSorbent Assay (ELISA) and Reverse Transcription-Polymerase Chain Reaction (RT-PCR). A total of 285 stool samples were collected from infants and children 0-5 years of age, who reported with diarrhoea in six different hospitals in Kano, Nigeria between November 2013 and July 2014. The diarrhoeic Stools were analyzed for RV antigen (ELISA) and the (RV) positive stools were further subjected to VP7 and VP4 genotyping using gene specific primers RT-PCR.

Results: Rotavirus was detected in 36.5% 104/285 of the diarrhoeic children. The infection occurred throughout the study period with higher peaks in the drier month of April 77.6% 38/49 and lowest in July 12.2% 5/41

Pearson Chi Square analysis: (X²= 27.720, P<0.05, df=1). The highest prevalence of RV infection was in children 41-50 months 50% 3/6 The RV was detected more in male 37.2% 61/164 than female 35.4% 43/121 children and no statistically significant difference was observed P>0.05. Three different rotavirus P-genotypes P8, P4, and P6 were detected in this study and P6 48% was the most commonly detected. Mixed infection were detected and consisted only of P8+P6. Six different G-genotypes were detected. The predominant genotype was G2 35.0% 36/103. The most common G and P combination was found to be G2P6 with 19.4% 20/103 frequency of occurrence. A single GNTP8+6 mixed combination of rotavirus strains was also detected during the study. Strains such as G6, G9 and G12 were also detected at low levels.

Conclusion: Rotavirus was found to be an important cause of diarrhoea in children 0-5 years of age in Kano, North-Western Nigeria.
Survey of Hepatitis B surface Antigen (HBsAg) prevalence and its risk factors among pregnant women at Bishoftu Hospital, Oromia Regional State, Ethiopia

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Background: Hepatitis B is a global public health problem and a major cause of liver disease, including chronic hepatitis, cirrhosis and liver cancer. Pregnant women with hepatitis B virus infection are reservoir for the virus and do have high potential to transmit it to their fetuses and newborns. The objective of the study was to investigate sero-prevalence of hepatitis B surface antigen and assess risk factors among pregnant women.

Methods & Materials: Institution based cross-sectional study was conducted from July to September, 2014 among a total of 202 pregnant women attending Bishoftu General Hospital. A pretested structured questionnaire was employed to collect data on socio-demographic and HBV infection risk factors. For Hepatitis B virus sero-marker analysis, blood sample was collected and tested for the presence of hepatitis B surface antigen using Enzyme Linked Immunosorbent Assay kit. Obtained data were evaluated by frequency, logistic regression analyses, and a significance level of 5 % (α = 0.05) was established.

Results: A total of 202 pregnant women were included in the study and 11(5.4%) of the study participants were positive for HBsAg. In the study, 84(41.6%) were completed their secondary school and majority (40.1%) of participants were in age group 25-29 years. Our finding revealed that none of the study participants were aware of their HBV sero-status. Sero positivity for hepatitis B surface antigen was statistically associated with history of abortion (AOR: 6; 95% CI :( 1.39-27.69); P-value: 0.017), surgery (AOR: 5; 95% CI: 1.04-24.31; P-value: 0.045) and family history for hepatitis (AOR: 11; 95% CI (1.63 80.44); P-value: 0.014).

Conclusion: According to the research findings, there was an intermediate endemicity of hepatitis B virus infection and history of abortion, surgery and family history for hepatitis were the major risk factors for the high prevalence of hepatitis B infection in the study. Therefore, pregnant women should be routinely screened for HBV and awareness creation on the mode of HBV transmission is recommended.
Memory B cell response to Japanese Encephalitis vaccination in JE endemic area of Uttar Pradesh
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**Background:** Japanese Encephalitis (JE) is the leading cause of viral encephalitis in Asia. Vaccination is the most effective countermeasure for protecting individuals from Japanese Encephalitis virus (JEV) infection. Neutralizing antibodies and its persistence after JEV vaccination is considered as important correlate of protection. Memory B cells play an important role in replenishing the pool of long lived plasma cells to maintain protective antibody titre. Recognition of these cells might be helpful in prediction of long term vaccine induced immunological memory. Therefore we utilized a B cell ELISPOT assay enumerate JE specific memory B cell in JE vaccinated children at different time points.

**Methods & Materials:** Children from Kushinagar District, a endemic area for JE in Uttar Pradesh were vaccinated under JE vaccination programme. Blood samples were collected from pre-vaccinated children followed by post-vaccination sequential sampling at day 10, 28, 56 and 6 month. PBMC were isolated and JE specific memory B cells (MBC) evaluated by B cell Elispot assay. Spots were counted and JE specific MBC were expressed as JE specific IgG Antibody secreting cell per 10^5 PBMC.

**Results:** Before vaccination (Day 0) JE specific MBC were at baseline although in few children memory B cells were detected this could be because of natural infection. On day 10, slight increase in count was observed. However significant increase in JE specific memory B cells was observe at 28 post vaccination (mean 36.97 spot forming cell per 10^5 PBMC; p<0.0001). On day 56, Decrease in MBC was observed (mean 11.08 spot forming cell per 10^5 PBMC, p< 0.0001). However, 6 month post vaccination this count was maintained (mean 11.59 spot forming cell per 10^5 PBMC) and found to be greater than baseline (Day 0).

**Conclusion:** This study enumerates persistence of memory B-cells after vaccination on JE endemic area. This will add to existing knowledge and contributes to designing of an improve vaccine against Japanese encephalitis. Role of memory B should also be evaluated as a potential component in a surrogate assay of vaccine effectiveness.
Practice of people in dealing with animals related to Crimean-Congo Hemorrhagic Fever in Nur County, Mazandaran Province, Northern Iran

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Background: Crimean-Congo Hemorrhagic Fever (CCHF) is a viral zoonosis tick-borne disease transmitted by tick biting, contact to blood or carcass of infected animals or humans. Iran is located in the category of countries with high prevalence of CCHF. However, no report of this disease was made from Mazandaran Province until 2010. Because of the first report of CCHF occurrence in Nur County, Mazandaran province in occupation related to animals, this study was undertaken to investigate high risk practice of people engaged in occupations related domesticated animal.

Methods & Materials: In 2012, a cross-sectional study was performed on 314 people including livestock farmers, animal keepers, shepherds, butchers, abattoir workers, chefs and veterinary staff to investigate their practice against CCHF disease in three Districts of Nur County. Prevalence of each practice including lack of protective wear, contact with livestock, slaughtering and contact with fresh flesh and blood of livestock and removing and squashing ticks from animals' body with unprotected hand were reported and relationship between each practice and demographic and ecologic variables were analyzed by Pearson's chi-square and binomial regression tests (P < 0.05).

Results: 289 out of 314 individuals were interviewed. Odds ratio (OR) of high risk practices including lack of protective wear when slaughtering or slicing fresh raw livestock meat was high in livestock farming (OR = 29.69, CI: 10.56-83.41), in people older than 59 years and more (OR = 23.93, CI: 3-190.8), in illiterate individuals (OR = 12.86, CI: 3.52-46.99) than other groups. Removing and squashing ticks from animals' body with unprotected hand was high in category of butchers who worked in sheep and beef husbandry than other occupations (OR = 40.5, CI: 5.37-305.34).

Conclusion: Our results proved high risk practices in the animal husbandry occupations; it would be a continuation of the increased risk of CCHF and even its epidemic among them.
Viral burden in acute respiratory tract infections in hospitalized children in the wet and dry zones of Sri Lanka

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Background: Acute respiratory tract infection (ARTI) is one of the most common acute illnesses of childhood. Mostly encountered viral etiology of ARTI in children under 5 years are respiratory syncytial virus (RSV), parainfluenza types 1, 2 and 3 (PIV), adenovirus (AV), influenza virus types A and B, coronavirus (CoV), human Boca virus (hBoV) and human metapeumo virus. (hMPV) This study was conducted to identify the viral burden in hospitalized children with ARTIs to map the occurrence of these viruses with local seasonality.

Methods & Materials: Nasopharyngeal aspirates (NPA) of inward patients (1 month - 5 years) with ARTI were collected in Teaching Hospital, Gampola (THG) and Teaching Hospital, Anuradhapura (THA) from March 2013 - August 2014. Following screening of NPA with indirect immunofluorescence assay (IFA) specific viral aetiology was detected by a direct immunofluorescence assay (DFA). IFA negative hundred NPA were tested for hMPV, hBoV and CoV. Viral seasonality and the overall viral burden were evaluated and the descriptive statistics was expressed using measures of central tendency.

Results: Out of 443 and 418 NPAs tested, RSV was detected 94 children (59.96 %) in THG and 85 children (51.51%) in THA. In both cohorts RSV was detected throughout the year. In the dry zone, the peak viral incidence was noted from May -July in 2013 and 2014. In the wet zone two peaks were observed: December-January in 2013 (major peak) and in April in 2013 and 2014 (minor peak). Period prevalence of RSV ARTI in THG was 4.7 % and in THA was 4.25 %. The RSV incidence at THG and THA was 31.3 and 28 /100000 person years. The hMPV distribution was similar to that of RSV.

Conclusion: Knowledge of seasonality of the occurrence of viral aetiologies in children with ARTI is important to implement early preventive measures, such as vaccination for influenza A, use of respiratory precautions and health education. Identifying the viral aetiology by proper virological diagnosis will reduce the empirical use of antibiotics and thus will contribute to reduce the cost and to prevent the emergence of anti-microbial resistance.
Cross-species transmission of mycobacterium tuberculosis in mahouts and captive elephants: Implications to health policy

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**Background:** There are nearly a thousand captive Asian elephants and not less than 3,000 mahouts in southern India. In the hands-on and open systems of captive elephant management, diseased mahouts and captive elephants could present the risk of cross-species tuberculosis transmission. With the help of evidence based results, we intend to formulate specific policy guidelines, which can suggest locally relevant preventive and control measures to help mitigate the risk of cross-species infection.

**Methods & Materials:** Over a period of three years, one time screening of nearly 800 elephants and their mahouts was achieved. Tuberculosis screening of mahouts was done by clinical examination, chest X-ray evaluation, sputum culture and tuberculin skin testing, as required. Screening of elephants was done using the USDA licensed serological test, DPP Vet Assay® (Chembio Diagnostics Inc., Medford, New York) and trunk wash culture, as required. Detailed contact investigation of traceable human and animal contacts of the identified diseased mahouts and elephants were done. We examined three different contexts of tuberculosis transmission among captive elephants and mahouts. First scenario is the risk of infection from an infected mahout to an elephant. Second is the risk of infection from an infected elephant to a mahout and third is the risk of infection from an infected elephant to another elephant.

**Results:** There is evidence to suggest cross-species tuberculosis transmission. However, under the tropical climatic conditions in southern India, the risk of infection to a captive elephant from a diseased mahout seems to far outweigh the risks of infection to a mahout or another elephant, from a diseased elephant. There are political as well as ethical consequences to the outcomes in each of the three scenarios and they are both varied and complex.

**Conclusion:** Mahouts and captive elephants in southern India are highly migrant and locating the subjects for contact tracing and follow-up testing is difficult. Hence, systematic and regular tuberculosis screening of mahouts and captive elephants is a challenge. Formulating as well as implementing policy guidelines for prevention and control of cross-species tuberculosis transmission, in the existing cultural and religious contexts of captive elephant managements in southern India, appears to be an even bigger challenge.
Serological survey of porcine cysticercosis and associated risk factors in pigs slaughtered at Ndumbuini abattoir in Nairobi, Kenya

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Background: Taenia solium is ranked as the most important foodborne parasite globally. Cysticercosis is caused by the larval stage of T. solium and is a serious public health risk in both rural and urban areas. Eastern and southern Africa have experienced a recent rapid growth in pig production, with increasing number of cysticercosis reports being received in concurrence with increasing smallholder pig keeping and pork consumption. However, there have been few studies undertaken in Kenya to investigate cysticercosis and more information is needed to assess transmission to humans.

Methods & Materials: This study was conducted in Ndumbuini, the main abattoir supplying pork to middle-low income consumers in Nairobi. The aims of the project were to: estimate the seroprevalence of cysticercosis; map the value chains associated with the abattoir; and assess the risk of cysticercosis-infected pork entering Nairobi food system. Blood samples were obtained from 700 pigs selected by systematic random sampling. Antigen ELISA was used to detect T. solium secretory antigen, while structured questionnaires, focus group discussions and observational tools were used to assess the risk factors.

Results: The overall seroprevalence of cysticercosis was estimated at 8.5% (95% CI: 6.4-10.7%). Pigs originated from 11 different counties. The prevalence rates ranged between 0-36%. Seven counties had seropositive pigs, four showed no positives. There was significant differences between sex (p=1) or age (p=0.13)of the pigs sampled. No positive cases were detected by post-mortem meat inspection. Therefore, all the carcasses were passed for human consumption. The abattoir had no facilities for treating Cysticerus solium infected carcasses.

Conclusion: This study highlights that T. solium is a public health risk to consumers of pork from Ndumbuini abattoir. We therefore recommend that meat inspection by palpation and incision be complemented with rapid tests. A wide scale general public sensitization should be undertaken.
Coagulase positive staphylococci and food poisoning toxins - A case study of an outbreak investigation occurred in a shepherd hut


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Background: Staphylococcal food poisoning (SFP) is an intoxication caused by the ingestion, with food, of emetic staphylococcal enterotoxins (SEs) produced by enterotoxigenic strains of coagulase positive staphylococci (CPS): especially *Staphylococcus aureus*. Staphylococci are commonly found in humans and in a wide variety of animals. The contamination of food with CPS is commonly caused by inadequate food processing methods or the use of mastitic milk. In case of staphylococcal mastitis of ruminants, *S. aureus* can be carried over from the udder into milk. Thus unpasteurized milk cheeses, contaminated by enterotoxigenic strains of staphylococci, may be involved in SPF outbreaks.

Methods & Materials: During August 2015 symptoms referable to SFP were described in a group of excursionists after eating raw milk cheese produced in a south tyrolean malga (shepherd hut). As consumption of cheese has been supposed to be a common risk factor, the case has been reported to the Local Veterinary Service that tried to rule out the source of the outbreak in cooperation with IZSVe-laboratory. Cheese sample, bulk tank milk and individual milk samples from the 41 dairy cows, rared at the malga, were collected. Food sample were analyzed for SEs type A, B, C, D performed according to the EU-RL screening method for CPS v5 (extraction followed by dialysis concentration and immuno-enzymatic detection). CPS were counted in cheese according to ISO 6888-2:1999. CPS were tested for SEs genes by PCR and biotyped for addressing host specificity by Italian National Reference for CPS.

Results: Mean value of CPS in cheese was 2600 cfu/g and presence of SEs was confirmed (test value > 2.20). *S. aureus* have been isolated from bulk tank milk and from 23.8% of individual milk samples. Just one of the 41 cows was reported with clinical mastitis. CPS were submitted to antibiogram without encountering any detectable resistance. *S. aureus* strains have been submitted to PCR test for genes encoding SEs.

Conclusion: Bacterial contamination of raw milk can occur even under optimal hygiene conditions, and the discrimination of the toxigenic nature of the strains can facilitate the management of potentially risky situations. Importance of SFP for public health justifies further investigation.
Rodent leptospirosis in North Khorasan Province, Northeast of Iran
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Background: Leptospirosis is a zoonotic diseases caused by leptospires, which is belonged to species of Leptospirainterrogans containing over 212 serovars. Rodents can act as a major source of infection for humans and animals. The disease has a global distribution, mainly in humid, tropical and sub-tropical regions. In Iran the disease is endemic in some parts of the north of the country.

Methods & Materials: This study was conducted to investigate the existence and important of Leptospira species in rodent of North Khorasan Province, Iran. For this study, thirty six rodents were trapped alive. Blood samples were taken and serum were separated and kept in freezer for serological investigation. The seropositiveserovars were identified and the antibody titers were measured by the standard microscopic agglutination test (MAT), using a panel of 8 strains of live Leptospira species as antigens. Serial dilutions (1/100 to 1/1600) of serum were used.

Results: The result of this study showed that 12 samples (33%) had a positive reaction against one or twosarovars. In general, 5 samples had a positive reaction with serovar Pomona, 3 samples with Australis, 2 samples with Tarasevian and 2 samples with Icterohaemorrhagiae. The results also showed that the most prevalent leptospiraserovar was Pomona (14%) and the most common titer was 1/100 (10 samples) and the highest titer was 1/200 (2 samples).

Conclusion: This study of leptospirosis is the first one in rodents in this region and it showed that Leptospirosis is prevalent in rodents in North Khorasan Province. However, further study is required to determine the importance of rodents in circulating the Leptospires into the animal and human populations.
Biosecurity risk of wild bird markets and wild bird trade to avian influenza in Kaduna State, Nigeria

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Background: Despite possible introduction of highly pathogenic avian influenza (HPAI) H5N1 virus into Nigeria by wild birds, few studies were undertaken on live wild bird markets (LWBM) role in HPAI surveillance. Study assessed LWBM biosecurity, sellers’ knowledge, attitude and practices (KAP) on biosecurity and wild bird trade (WBT) in Kaduna State.

Methods & Materials: LWBM biosecurity and sellers’ KAP assessed using biosecurity checklist and structured questionnaire respectively. Wild bird trade studied through market survey.

Results: All sellers were male; some (22.7%) report sick birds only when attempted treatment fails. Sellers kept poultry at home (78.9%); encouraging (100%) poultry–wild birds contact. Over 31.6% sellers don’t wash hands with soap after handling birds. No seller knew any HPAI clinical sign though 21.1% knew HPAI affects human beings and none believes HPAI affects human beings. Sellers would report HPAI outbreak to reduce losses (38.9%). None of the LWBM was fenced with birds tied and allowed to move in 25% of LWBM. Cages were wood/metal while fenced pens constructed from wood/wire mesh with un-cemented floor. No LWBM sourced birds from one reliable source neither were birds separated by species. Other livestock were sold in 75% of LWBM. Free flying birds interact with wild birds in 75% of LWBM while local poultry–wild bird interaction occurred in 25% LWBM. No seller wore protective clothing. All LWBM clean cages regularly though none used disinfectant with 25% sellers disposing manure improperly. Over 75.9% of biosecurity features in LWBM were risky with 76.2% being risky biosecurity practices and 80% (17/21) due to poor LWBM infrastructures. Food (31.8%), traditional medicine (45.5%); pets (77.3%) were reported wild birds uses. There was high demand for birds of prey during election years. White stork (11.42%) and gerese (9.94%) were the main birds on sale. Threatened and rear wild bird species were being traded in the LWBM. Over 45% of birds were sourced from 9 foreign countries with majority coming from Chad.

Conclusion: Sellers’ KAP was poor with low risk perception. Biosecurity in LWBM in Kaduna State was poor. Kaduna State WBT is linked to the global trade and could be a source for disease introduction into Nigeria. Trade be legalized and regulated; sellers trained on biosecurity and routine surveillance in LWBM.
Ecoepidemiology of Rickettsia parkeri in the Paraná Delta, Argentina

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Background: In South America, several cases of human rickettsiosis caused by Rickettsia parkeri were documented in Uruguay, southern Brazil and the Paraná River delta of Argentina. There, the main tick vector is Amblyomma triste. Adults of A. triste seek blood meals from large mammals (including humans), whereas immature stages feed on small rodents.

Methods & Materials: With the aim of shedding light on the ecology of this emerging disease, we conducted field studies at sites of the Paraná River delta, which consisted of systematic collection of ticks and blood samples from rodents (Fig. 2) and cattle, and also questing ticks from the vegetation. Sampling sessions were carried out monthly during 2011 and 2012 at 16 points that differed in their exposure to cattle and vegetation type (natural or implanted forest).

Results: Prevalence of infection in adult questing ticks was high (20.4%). Interestingly, the distribution of R. parkeri infection intensity observed in A. triste ticks was distinctly bimodal, with approximately 60% of the infected ticks presenting high rickettsial loads (Fig. 3). Questing ticks were more frequently found in natural grasslands than in implanted forests, and prevalence of infection were greater in those from grasslands (26%) than in forested areas (8.3%). The dominant rodent species were Akodon azarae and Oxymycterus rufus. In both, the seroprevalence to R. parkeri was greater in those captured in grasslands than in implanted forests. The presence of cattle had a significant positive effect on the burdens of ticks on rodents and the abundance of questing ticks in the vegetation. Most cattle (90%) were seropositive, and the seasonality of the titres of antibodies against R. parkeri matched that of the tick infestation on cattle.

Conclusion: The risk of human exposure to R. parkeri infected ticks in the Paraná River delta is high. Our results suggest that the silvopastoral activities that are on the rise in the region affect the dynamics of infection of R. parkeri. Cattle appear to favour the occurrence of the pathogen, whereas forestation seems to reduce it.
A survey of human and animal casualties resulting from bites of stray dogs in the municipal area in Palakkad district, Kerala

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**Background:** Recent estimates suggest there are nearly 1,197 stray dogs in the municipal area of Palakkad district in Kerala. It is also estimated that when no stray dog control measures are undertaken by the various civic bodies, there could be up to 25 per cent yearly increase in the numbers of stray dogs. Increasing numbers of stray dogs pose significant safety threat to both humans and domestic livestock and hence, mass killings of stray dogs by the public happen at times. At the same time, in recent years, there has been a huge outcry in the social media, especially by the various animal welfare organisations, against such killings of stray dogs in Kerala.

**Methods & Materials:** We intended to examine the magnitude of threat to public safety from the bites of stray dogs in the municipal area in Palakkad district. For the same, we undertook a survey and collected data from print and visual media on all reported cases of stray dog bites from January 2015 till date. We also undertook one-to-one questionnaire surveys with medical and veterinary doctors working with medical and animal husbandry department in the area and the general public.

**Results:** Over the last ten month period, nearly 3800 humans and 459 domestic animals were reported to have suffered stray dog bites in the municipal area. In humans, males (60\%) suffered more bites than females (40\%). Compared to humans, in animals, the number of reported bite cases is severely underestimated, mainly because of the poor surveillance systems for recording animal casualties. Most of the reported animal cases were in domestic goats and only a lesser number were in domestic cows and dogs. In addition, two cases of deaths in humans and eight cases of deaths in animals, which were attributed to rabies virus infection had history of stray dog bites. Among the animal deaths due to rabies, there were five goats, two cows and one domestic dog.

**Conclusion:** Unless the local civic bodies undertake adequate measures to control the numbers of stray dogs, changing the public perception as well as opinion against the mass killings will remain an uphill task.
Predominance of "atypical" enteroaggregative escherichia coli among human, animal, foods and associated environmental sources

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Background: Infectious diarrhoea particularly due to pathogenic bacteria is a major health problem in developing countries, including India. Despite significant reports of diarrhoeagenic Escherichia coli (DEC) pathotypes across the globe, there is paucity of studies which reveal their relatedness with respect to their isolation from different sources. This present study determines isolation and identification of DEC pathotypes from different sources, their genetic characterization and antibiogram sensitivity profiling.

Methods & Materials: A total of 336 samples comprising of diarrhoeic stool samples from infants (n=103), young animals (n=106), foods and associated environmental sources (n=127) were screened for E. coli. The identified E. coli were confirmed as DEC pathotypes by using PCR based assays. These isolates were further studied for their genetic diversity using Pulse Field Gel Electrophoresis (PFGE) subtyping tool and their antibiogram profile was determined against seven commonly used drugs.

Results: Of the four DEC pathotypes investigated, Enteroaggregative E. coli (EAEC) was found to be the predominant pathogen with an isolation rate of 16.50% from infants, 17.92% from young animals and 10.24% from foods and environmental sources. These EAEC isolates, on further characterization revealed predominance of 'atypical' EAEC, with an isolation rate of 10.68% from infants, 15.09% from young animals and 10.24% from foods and associated environmental sources. On PFGE analysis, discrimination was also evident within DEC pathotypes, as only closely related EAEC isolates clustered together irrespective of their source of isolation. Further, higher antibiotic resistance pattern was observed among the isolated DEC pathotypes as almost 86.44% of isolates were found to be resistant against ≥3 tested drugs.

Conclusion: EAEC pathotype in particular ‘atypical’ strains were found to be the predominant pathogen. On PFGE analysis, sharing and circulation of EAEC isolates between human and animal, including foods and associated environmental sources was evident. Besides this, an alarming antimicrobial resistance profile was observed for majority of the recovered DEC pathotype isolates.
Development of safe, effective and immunogenic vaccine candidate for diarrheagenic Escherichia coli main pathotypes in mouse model
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Background: Glanders is a highly contagious and fatal zoonotic disease primarily of horses, mules and may occur in felids in wild fields. At once time, B.mallei infection occurred worldwide, but over last 100 years occurrence decreased and many parts of world has been eradicated. However, sporadic cases of glanders are still registering in Far East, South America, North Africa, Middle East and Asia. In Mongolia, major glanders outbreaks were reported during the middle of last Century and due to specific measures, the prevalence was decreased to 0.05% by the end of 1980s. During the last decade, has been detecting sporadic cases and glanders likely re-emerging in Mongolia. The present study aimed to conduct the risk-based survey and identify B.mallei by conventional and molecular methods and identify a course of re-introduction of B.mallei.

Methods & Materials: Risk-based survey was conducted in Central part of Mongolia from 2014 to 2015. A total of 809 horses were tested by mallein test and CFT. Tissue samples from positive reactors were subjected for bacteriological examination and isolates were characterized by conventional and molecular methods, such as PCR, immunohistochemistry and etc. PCR performed using primers based on nucleotide difference in the 23S rDNA between B.mallei and B.pseudomallei, described previously elsewhere.

Results: Several cases of glanders, with clinical symptoms were reported and were conducted risk based surveys in outbreak areas. Of the 809 horses, 9 were positive by mallein test and 12 by CFT. From pathological samples were isolated 6 Gram negative, nonmotile rods suspicious for B.mallei. These isolates, by PCR with primers specific for B.malei and B.pseudomallei were positive, and with B.mallei specific antisense primer, sense primer specific for B.cepacia, B.vietnameses, B.mallei and B.pseudomallei and competitive oligonucleotide probe for other Burkholderia, except B.mallei were positive for B.mallei. Beside of these, in Gimza stained tissue sections were observed beaded bacterium, by immunohistochemistry were detected B.mallei.

Conclusion: Equine glanders cases, with clear symptoms, isolation and identification of B.mallei are indicating that glanders is re-emerging with potential risks on public health in Mongolia.
Mozambique experience in implementing One Health Surveillance as an innovative tool to understand the risk of spillover of emerging and zoonotic infections between wildlife and humans

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**Background:** Zoonotic diseases are in the forefront position of emerging diseases, accounting for 70% of emerging diseases worldwide as a consequence of rapid deforestation, intense globalization, unplanned urbanization and global warming. Mozambique is a vast country, where rapid and unplanned urbanization is common, posing the risk for spill over of diseases from animals to humans. In 2012, Ministry of Health in collaboration with Faculty of Veterinary from Eduardo Mondlane University and Biotechnology Center established the first one health sentinel site to conduct research and surveillance in the interface between wildlife and humans.

**Methods & Materials:** The One Health sentinel surveillance site was established in Caia District, a rural area situated in Zambeze valley in the central part of the country. This district was selected because of the following characteristics, i) intense contact between humans and wildlife, ii) high vulnerability for flooding, iii) abundance of domestic animals such as cattle, pigs and poultry, and also abundance of breeding places for mosquitoes. The one health sentinel surveillance in Caia comprised three key pillars, i) surveillance of zoonotic diseases in febrile patients attended at the local district hospital, ii) surveillance of zoonotic diseases in cattle, pigs, poultry and micro mammals (bats and rodents) and iii) entomologic investigation mostly in mosquitoes and ticks.

**Results:** We assessed the following indicators: number of post graduation students involved, number of projects initiated, number samples collected, number of report generated and number of manuscripts published. A total of 2 PhD students and 4 Msc students from different disciplines, such as entomologists, biologists, veterinarians, medical doctors and epidemiologists are conducting their thesis in this site. An estimated 1000 serum samples were collected from febrile patients. Mosquitoes were collected as part two PhD thesis. Two project that will investigate zoonosis in poultry and cattle will start in February 2016.

**Conclusion:** After two years of implementation of this project, we conclude that establishment of One Health surveillance sites represents a strong platform to conduct transdisciplinary research combining human, veterinary and entomological data, so that to improve our knowledge on the risk of spillover of zoonotic diseases.
Study of antibody dynamics in horses vaccinated against West Nile Virus (WNV)

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**Background:** WNV is an RNA virus belonging to *Flaviviridae* family, transmitted by mosquitoes, causing zoonosis. Humans and horses are dead-end hosts. To date, there is no cure for the disease. The prevention can be achieved minimizing the exposure to the vector or through vaccination in equine species. In Italy, two vaccines are authorized: the “Equip WNV - Pfizer” (inactivated vaccine, VM-2 strain) and the “Proteq West Nile - Merial” (recombinant canarypox virus, vCP2017 strain, that expresses the WNV prM/prE genes). Both vaccines protect against WNV lineages 1 and 2 strains. No vaccination is available for humans. Aim of this research was the study of the dynamic of antibodies in sera of vaccinated horses.

**Methods & Materials:** Two groups, each consisting of 20 healthy horses, serological negative to WNV, were submitted to vaccination (booster after 28 days) using authorized vaccines. After vaccination, horses were examined to evaluate the immune response from 0 to 365 days after vaccination (DAV). IgG were detected through the kit ELISA: ID Screen West Nile Competition Multi-species – ID.vet. IgM were detected using the kit ELISA: West Nile Virus IgM Antibody Test - IDEXX. All sera were tested by serum neutralization (SN) test according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2013).

**Results:** Data relating to IgG response showed that Pfizer vaccine induced an earlier immune response compared to the Merial one (100% of positive animals at 18° vs 38° DAV). Both vaccines produced appropriate levels of IgG for one year. SN results showed that Merial vaccine stimulated long-lasting and more intense response compared to Pfizer one (65% vs 21%). Horses treated with Merial vaccine had high neutralizing antibody titers for one year unlike of subjects vaccinated with Pfizer. All horses vaccinated produced IgM.

**Conclusion:** Both vaccines gave adequate antibody titers. Data suggest to use Pfizer product during outbreaks thanks to its capacity to produce antibodies early, instead Merial vaccine might be used during prophylaxis plans. Both vaccines induced IgM production, therefore, DIVA (Differentiating Infected from Vaccinated Animals) strategy is not applicable. This study can be useful as model to develop the indirect prophylaxis in humans.
Endemic toxoplasmosis and listeriosis in the perspective of 'The problem of shelter dogs' in Istanbul

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Background: Nowadays it is known that animal sourced infections may have serious threats against human health. It is a fact that Toxoplasmosis and Listeriosis are significant zoonotic infections and dogs play a substantial role on the prevalence of these infections. In our study, we have aimed to determine the seroprevalence of Listeriosis and Toxoplasmosis among shelter dogs in different animal shelters around Istanbul and to describe the role of dogs in the transmission of these zoonoses.

Methods & Materials: Blood samples from 100 dogs were collected and Tag-Man probe based Real Time PCR (qPCR) analyses of the samples were conducted regarding to the high sensibility and characteristics of this technique and the results were evaluated according to the gender and age of the dogs. Real Time PCR (qPCR) analyses were conducted using specific primers and probes that target the gene regions 529 bp RE for Toxoplasma gondii and Listeriolysin O (hyl A) for Listeria monocytogenes.

Results: According to our results, it is found that 19 dogs (19%) out of 100 are T. gondii positive, and 12 dogs (12%) are L. monocytogenes positive. It is seen that seropositivity among the 0-2 age group is high in both zoonoses and also according to gender L. monocytogenes is high among the females and T. gondii is high among the male dogs.

Conclusion: When compared to other cities in Turkey, it is found that our results in Istanbul province have a lower prevalence. Beside this, we think that these results may be a serious risk for the people living in this city and optimal protective cautions should be taken. We estimate that our study will contribute the data about the prevalence of these zoonoses not only in our country but also all around the world.
Brucellosis presenting as mediastinal lymphadenopathy with raised β2 microglobulin

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Background: 75 year old male with high grade continuous fever for past one month. It was associated with rigors, chills and sweating. Clinical examination was unremarkable and routine screening for pyrexia was unrewarding. CECT revealed mediastinal lymphadenopathy. Brucella serology was positive with dilutional titers of 1:1280. β2 microglobulin levels were high i.e 3570 i.u. The patient was treated with Inj Streptomycin, Cap Rifampcin and Cap Doxycycline. His symptoms resolved and was followed for 11 months.

Methods & Materials: Brucellosis is a zoonotic disease presenting mainly as fever, sweating, low back ache and ill health. Its incubation period varies from weeks to months with fever persisting and undulating. Clinical signs include anaemia, arthritis, cervical lymphadenopathy, hepatosplenomegaly. Our patient presented with classical undulant fever and had mediastinal lymphadenopathy which is relatively rare manifestation of brucellosis. In addition our patient had low back ache with anaemia which prompted us to screen him for malignancy especially multiple myeloma. His β2 microglobulin levels were high and brucella serology at higher dilutional also came high positive. Patient was started on brucella treatment protocol and marked improvement was noticed at 4 weeks of completion of treatment. Patient has been followed for next 11 months and was doing well. The aim of presenting this case is that mediastinal nodes can be isolated involved in brucellosis and high β2 micoglobulin levels should not preoccupy the clinical decision for multiple myeloma.

Results: Our patient presented with PUO and initial brucella serology was negative. The CT chest revealed mediastinal lymphadenopathy with borderline rise of LDH and high levels of β2 microglobulin. We considered lymphoma as a cause of his fever. As this patient developed backache and headache, repeat brucella serology was sent and titer came 1:640. This titer doubled in a weeks time. He was put on brucella treatment.

Conclusion: The present case gives dimension to varied presentations of brucellosis. In particular brucellosis should be kept as a strong differential diagnosis of PUO in a endemic zone despite non encouraging brucella serology results . We advocate serial serology testing should be performed before contemplating invasive procedures for establishing cause for PUO.
Clinical spectrum of melioidosis at a tertiary care hospital in South India

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Background: Melioidosis, caused by the Gram-negative bacillus Burkholderia pseudomallei, is an infectious disease of public health and a biothreat importance. Clinical presentations of melioidosis are protean, hence, called a remarkable imitator. The number of cases of melioidosis reported in India is considered to be the tip of an iceberg as several cases may not be diagnosed due to lack of awareness among the clinicians about the infection and the laboratories not capable of isolating and identifying B.pseudomallei.

Methods & Materials: It is a retrospective study and data was collected from the in patient case records of patients with melioidosis from 2004 to 2014. We report the clinical features and outcome of cases of this unusual infection at our institute, a tertiary care hospital in South India.

Results: There were 17 cases of melioidosis from our Institute over last 10 years. Majority of the patients were agriculturists by occupation. Diabetes mellitus was the underlying comorbid condition in all patients except in one case. Twelve out of 17 cases (70.59%) presented with sepsis. Six cases (35.29%) had fulminant course and succumbed before the microbiology reports arrived and definitive treatment is started. Skin lesions were apparent in 10/17(58.82%) cases. Seven out of 17(41.18%) cases had deep-seated abscesses involving the liver, spleen, prostate, brain, bones and joints. Thirteen cases out of 17(76.47%) had pneumonia. In all the cases, B. pseudomallei was isolated from the blood using the BacT/alert (bioMerieux) system, within 24 hours. All the isolates were sensitive to co-amoxiclav, co-trimoxazaole, ceftazidime and carbapenems. The distinctive pattern of susceptibility, to TMP-SMX was an important clue in the identification of B.pseudomallei. Eleven patients out of 17 received appropriate antibiotic therapy based on the susceptibility reports, by 4th day of hospitalization. The initial intensive regime consisted of intravenous ceftazidime for 2 weeks or imipenem or meropenem for 14 days followed by oral TMP-SMX (320/1600 mg/kg 12th hourly) for 3-6 months.

Conclusion: Pneumonia was the most common presentation of infection by B.pseudeomallei. Diabetes is present in 94% of cases of melioidosis presenting to our institute. Mortality was observed in 6 (35.29%) cases.
Study of clinical, laboratory abnormalities and outcome in patients with scrub typhus at a south Indian tertiary care hospital

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Background: Scrub typhus is caused by Orientia tsutsugamushi (O.tsutsugamushi) an obligatory intra-cellular gram negative bacterium, a major cause of febrile illness in Asia pacific region.

Methods & Materials: This is prospective observational study on patients with fever with Weil felix ox-k positive in 1:320 dilutions or more or IgM ELISA and admitted between August 2014 to August 2015 in general medicine wards and emergency unit, Nizam's institute of medical sciences, Hyderabad. Febrile illness due to other established causes (dengue fever, enteric fever,malaria, infective endocarditis, bacterial meningitis and culture positive fevers) even when co-infection present were excluded from the study.

Results: A total 71 patients included in this study, mean age was 43.77 years. Majority of patients belong to agricultural background(35.2%). The mean duration of fever before presentation to our institution was 12 days. Most common symptom was cough 33.8%. Breathlessness was seen in 31%, vomitings in 29.65%, headache in 28.2%, altered sensorium in 8.5%, seizures in 1.4% and jaundice in 8.5% patients. Clinically signs of pneumonitis were seen in 7%, ARDS noted in 68.5%, pleural effusion in 3 (4.2%), hepatosplenomegaly in 15.49% patients. Pallor was noted in 16.9%, pedal oedema in 8.5%, icterus in 8.5%, lymphadenopathy in 9.9%, rash in 8.5% and eschar in 7% patients 57.75%. Pallor was noted in 16.9%, pedal oedema in 8.5%, icterus in 8.5%, lymphadenopathy in 7(9.9%) patients, rash in 6(8.5%) patients, eschar in 7% patients. Bradycardia was observed in 1 patient, tachycardia in 50.7% patients. Hypotension was found in 5.6% patients. Elevation of transaminases was seen in 83.1%, serum alkaline phosphotase in 63.38%, bilirubin elevation seen in 50.7% patients. Severe hypoalbunemia was seen in 47.88% patients. Acute kidney injury was seen in 14.1%. Complete cure was seen in 97.18%.

Conclusion: Majority of our patients with scrub typhus are farmers. Pulmonary symptoms are the most common manifestation. Hepatitis is the most common laboratory abnormality in our study. Mortality is low with prompt treatment.
Camel's milk as a source of human toxoplasmosis in Butana area - Sudan

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Background: Toxoplasma gondii is widely distributed in most parts of the world, affecting animals and humans. Toxoplasmosis is considered the third cause of death associated with food-borne infections in Europe (EFSA, 2007) and USA (Mead et al., 1999). Milk was implicated as a source of Toxoplasma gondii infection in several reports (Jackson and Hutchison). Bonametti et al., described toxoplasmosis in breast fed child whose mother had acquire toxoplasmosis by ingestion of raw goat milk. Manal et al.(2005) reported a prevalence of 61.7% in Sudanese camels. The aim of this study is to improve the role of naturally infected camel's milk as a source of human toxoplasmosis in Butana area - Sudan.

Methods & Materials: Ten milk samples were collected from infected camels at Butana area (Eastern Sudan). The infection was confirmed by IgM anti-T. gondii ELISA test, using a commercial kit (DRG Instruments GmbH, Germany). For each milk sample, two naive kittens (3-week-old) were fed with 10 ml for each and four Albino mice were inoculated by gavage, 2 ml per sample for each mice. For each batch of samples inoculated, one kitten and three mice were kept as control and not inoculated. ELISA test was carried out on the survived infected kittens and mice sera. Fecal samples from kittens were examined daily for oocysts detection. An autopsy was carried out on mice and tissues were fixed in 10% formalin for histopathology to detect Toxoplasma tachyzoites or cysts.

Results: All infected kittens began to shed Toxoplasma oocysts 3-5 days post infection. Infection was documented in all infected kittens and 21 of the 23 surviving mice by positive serology (ELISA) results. 17 mice were died at 3-14 day post infection. Toxoplasma tachyzoites were observed in the survived mice specimens. Non of the control kittens or mice showed evidence of infection by ELISA test.

Conclusion: The excretion of Toxoplasma gondii tachyzoites in camel's milk documented in this study and the high sero-reactivity of Toxoplasma in camel’s herders in Butana area (100%) reported previously by Khalil (2004), warrant a closer look into its public health significance among Sudanese nomads who consume raw camel's milk.
Survey for avian influenza and Newcastle disease antibodies and viruses in domestic and wild birds in Bauchi and Gombe States, Nigeria

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Background: Avian influenza (AI) infected and spread to 97 Local Government Areas affecting 25 of the 36 States of Nigeria. Virulent form of Newcastle disease has assumed endemic status in Nigeria. The study was conducted to detect the prevalence of H5 and H7 AI viruses and virulent strains of ND virus in poultry and wild birds in Bauchi and Gombe States, Nigerian.

Methods & Materials: A cross-sectional study was conducted by collecting and testing cloacal swabs and blood of 910 apparently healthy domestic and 90 wild birds in randomly selected households and farms. Poultry species, captive and free flying wild birds were sampled in the study. All sera tested for the presence of H7 AI antibodies using (ELISA) and (HI) tests were negative.

Results: Of the 500 sera tested from each state for AI H5 antibodies using (HI) and (ELISA) tests, 30 and 28 were positive in Bauchi and Gombe States respectively with a higher (16) detection rate by (HI). Thus AI H5 seroprevalence of 4.6% for both states and 3.2% and 4.9% for Bauchi and Gombe states respectively were obtained. The assessment of ND status showed seroprevalence of 36% in Bauchi State and 47% in Gombe State with an overall seroprevalence of 38.5% in both states. The highest specific prevalence of ND were 61.5% in Bauchi north and 57.6% in Gombe north. Chickens held the highest seroprevalence of AI H5 (3.2%) followed by turkeys (0.6%), guinea fowls (0.2%) and pigeons 0.2%. Poultry in live bird markets had a 1.6% specific seroprevalence, 3.0% was obtained for commercial poultry and 2% for free ranging poultry. Molecular detection of NDV using PCR gave an overall prevalence of 17% with the prevalence of 8.5% in poultry and wild birds respectively. Local chickens held 10% of the total ND prevalence.

Conclusion: Avian influenza H5 and ND antibodies and viruses were detected at significant levels in apparently healthy domestic and wild birds. Strategies for effective AI and ND control measures should therefore incorporate AI and ND routine and sustainable surveillance in domestic and wild birds held in households, commercial farms and LBMs.
Cutaneous anthrax in the Rostov region of Russia: Difficulties in the clinical diagnostics

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Background: Sporadic cases of anthrax in humans are recorded in Russia every year (2-11 cases per year within 2012-2014 years).

Methods & Materials: We provide description of 2 cases with modification of classical clinical picture, which led to difficulties in the diagnosis in the early stages of anthrax.

Results: In August 2014, 5-7 days before onset of symptoms 2 shepherds butchered fallen cow on a private farm in the Rodionova-Nesvetayevsky district of the Rostov region. In first patient the disease began with the appearance of four ulcers on the both forearms and left shoulder. When the ulcer were covered with a dark brown crust, the patient several days had carried out self-treatment. He tore the crust repeatedly and treated the wound with 1% iodine solution and brilliant green solution. Ulcers were painless. Common condition and temperature were satisfactory and he continued to work. After 1 week the left hand became significantly swollen and patient for the first time sought medical care to the district hospital. The surgeon diagnosed cellulitis and performed an autopsy and drainage of the wound on the left forearm. Anthrax was suspected only on the next day.

The patient was transferred to the infectious department of city hospital in Rostov-on-don, where anthrax was confirmed by PCR in samples from ulcers.

At the same time the second shepherd sought medical care in the district dermatologic hospital with several ulcers on his hands, subfebrile temperature and mild intoxications and was hospitalized with generalized pyoderma.

After confirmation of anthrax in the first patient, the second patient was transferred to the infectious city hospital. It was the 8th day of disease. He had 10 ulcers without edema on his fingers and forearms at the time of admission in the infectious department. They were covered with a black crust and surrounded with erythema and vesicles. Anthrax was also confirmed in the second patient by PCR in samples from ulcers.
Anthrax ulcer on the palm (2th patient)

**Conclusion:** Self-treatment, the late appearance of edema in the first patient and absent of edema of the extremities in second patient at the time of reference for medical care led to wrong diagnosis in clinical departments of non-communicable diseases.
Reducing vulnerability to the threat of Japanese encephalitis transmission in high risk districts in Nepal
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Background: Japanese Encephalitis (JE) is a vector borne viral zoonotic diseases, transmitted by Culex mosquitoes. It is main public health problems in Asian Countries including Nepal, engendering poverty and intellectual standard of nation hampering prosperity and misfortune of economy of life. People generally migrate from rural to urban areas for sustaining economy. To alleviate poverty, they rear pigs. Pigs are the reservoir hosts for JE virus. But, they have lack of knowledge about JE. Hence, the study was conducted with aim to study knowledge, attitude and risk factors associated with JE among pig farmers in Kathmandu, Nepal.

Methods & Materials: A cross sectional study was conducted in Kathmandu, Morang, Kapilvastu and Rupendehi districts of Nepal. Study was carried out from 2011-2013, based on eco-health approach, through biological sampling, questionnaires and focus group discussion. A total of 515 pig sera samples as well as 365 human sera samples were randomly collected from four project districts for JE survey. Three scale data were collected from existing source and analyzed by SPSS and GIS software for cluster analysis.

Results: Exposure to JE risk factors was common across pig farm and pig farming districts but with significant district level difference in knowledge and practice related to on farm JE risk reduction. JE vaccine up take was almost nonexistent (1/400) and mosquitoes control steps were uncommon across all four district. Spatial distribution of JE and Acute Encephalitis Syndrome (AES) seemed to be similar and amount of irrigated land use density and degree of landscape mixing with irrigated areas were positively associated with JE and AES. The human and pig sero-positivity were found to be 11.17(41/367) and 28.93 (149/515) in all four district of Nepal

Conclusion: The individual, community and geographic scale using a mixed methods approach allowed us to reveal the social drivers of Japanese Encephalitis, and revealed the need to train health professionals to look to collaborative efforts to manage these social and geographic drivers rather than to consider more classical medical interventions as the primary strategy against JE and other vector-borne diseases.
Is ocular dirofilariasis an emerging zoonoses in India? Report of five cases and review of literature

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Background: Dirofilariasis is a zoonotic filarial nematode infection, occurs occasionally in humans and humans are accidental dead-end hosts. Human infection by *Dirofilaria* species have been reported from various regions of the world mainly from Europe, Africa and Asia. Number of dirofilaria cases are gradually increasing in India. In the present study we report 5 cases of ocular dirofilariasis and review of published literature from India.

Methods & Materials: Medical and microbiology records of 5 patients with ocular dirofilariasis presented between May 2014 and September 2015 reviewed. *Dirofilaria* were identified morphologically based on size, body cuticle and prominent musculature. Systematic review of literature concerning dirofilariasis reported from India was performed.

Results: Four of five patients were males and the age ranges from 42 years to 60 years. In two patients the worm was extracted from sub conjunctival space, two patients the worm was extracted from nodule of lower lid margin and in one from the upper lid. In all the five patients the worm was identified as *Dirofilaria repens* based on morphological features. Microfilaria were not detected in peripheral smear and eosinophilia was absent in all 5 patients. Review of literature results revealed that in India, majority of dirofilariasis cases are ocular (>90%). Ocular dirofilariasis was reported from Kerala, Karnataka, Tamil Nadu, Delhi, Maharashtra, Gujarat, Assam, Haryana and Telangana (Present study cases) states and highest number from Kerala. *D. repens* (99%) is the most commonly isolated species from ocular dirofilariasis cases in India and rarely *D. tenuis*. The different sites of ocular involvement include periorbital, orbital, subconjunctival, subtenon and anterior chamber. The diagnosis was done by identifying the excised worm and no microfilaria were detected in peripheral smears of patients with ocular dirofilariasis. None of the patients showed eosinophilia. Surgical removal of the worm was definitive choice of treatment and prognosis was usually good in ocular dirofilariasis patients.

Conclusion: To the best of our knowledge, this is the first report of ocular dirofilariasis cases from Telangana state. In India, ocular dirofilariasis is on rise and awareness among ophthalmologists, microbiologists will improve the patient care.
Incidence of brucellosis in Livestock in North-Eastern India

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**Background:**
Brucellosis still remains a major endemic disease in India with considerable public health and economic significance. To understand its epidemiological status in livestock distributed in Northeastern India particularly Meghalaya, seroprevalence study based on ELISA and RBPT was undertaken from October 2012-September 2015, along with molecular characterization of the isolates.

**Methods & Materials:** A total of 4371 serum samples were collected from cattle (n=1505), buffalo (n=21), pig (n=2564) and goat (n=281) scattered over Meghalaya (n=3310), Manipur (n=404), Nagaland (n=389), Mizoram (n=100), Tripura (n=107), Sikkim (n=40), and Arunachal Pradesh (n=21). Besides, from Meghalaya, clinical samples viz. vaginal swabs, vaginal discharge of cattle (n=70), goat (n=1), joints aspirate from cattle (n=2), tissue samples such as placenta and uterus of cattle (n=3) and swine (n=2) and blood samples of cattle (n=157) and swine (n=17) were processed for isolation and identification by standard protocols, PCR and sequence analysis.

**Results:** Overall, Meghalaya represents a seropositivity of 5.6% by ELISA and 2.8% by RBPT. None of the samples from the remaining states except Manipur (0.49%) were seropositive by ELISA. Species-wise seropositivity ranged from 0% to 11.29%, with 11.29% for bovine and 0.78% swine by ELISA and 5.91% bovine and 0.15% swine, were positive by RBPT. Bubaline and caprine samples were found negative by both the tests. Analysis of variance in incidence of brucellosis among age groups and sex revealed no significant difference in mean sample positivity. From the clinical samples nine *Brucella* isolates was recovered *i.e.* seven from cattle, one each from goat and swine by culture and isolation. Further, AMOS and Bruce ladder PCR assay could confirmed the cattle and goat isolates as *Brucella abortus* and the swine isolates as *Brucella suis*. Bidirectional sequencing and BLAST analyses also confirms the cattle isolates as *Brucella abortus*. In addition, 54 cattle and 6 swine blood samples were found positive for *bcsp* gene encoding the surface protein of *Brucella*.

**Conclusion:** This study underscores the incidence of brucellosis only in Meghalaya and Manipur. Interestingly, in Meghalaya seropositivity was noted primarily in adjoining areas of Assam. Thus, it is imperative to develop a sustainable control strategy for timely intervention and further prevention.
Mixed infection of bovine tuberculosis and paratuberculosis in domestic livestock species of North India

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Background: Bovine tuberculosis (caused by *Mycobacterium bovis*) and paratuberculosis (caused by *Mycobacterium avium* subspecies *paratuberculosis*) are the most significant mycobacterial diseases of animals; causing huge economic losses to livestock production and also having significant impact on human health, worldwide. In India, limited information is available about the status of bovine tuberculosis and paratuberculosis in domestic livestock species. Present study was aimed to investigate the status of mixed infection of bovine tuberculosis and paratuberculosis in domestic livestock species of North India.

Methods & Materials: In present study, a total of 121 fecal samples were collected from Cattle (n=45), Buffalo (n=26), Goat (n=32) and sheep (n=18) of farmers herds of various agro-climatic conditions of north India and screened for the presence of acid fast bacilli and mycobacterium species using microscopy and direct PCRs, respectively. Primers specific to IS1081 and IS900 sequences were used in direct PCR for the identification of *Mycobacterium bovis* and *Mycobacterium avium* subspecies *paratuberculosis*, respectively.

Results: Of the 121 fecal samples, 35 (28.9%), 14.0, and 16.5% samples were positive for the presence of acid fast bacilli, *Mycobacterium bovis* and *M. avium* subspecies *paratuberculosis* using microscopic examination, IS1081 PCR and IS900 PCR, respectively. Species-wise, 26.6, 7.6, 28.1, 0.0% and 24.4, 7.6, 21.8, 0.0% samples from cattle, buffalo, goat and sheep were positive for the presence of acid fast bacilli, *Mycobacterium bovis* and *M. avium* subspecies *paratuberculosis* using microscopic examination, IS1081 and IS900 PCR, respectively. Of the 121 animals, mixed infection for *Mycobacterium bovis* and *M. avium* subspecies *paratuberculosis* was found in 9 (7.4%) animals. The presence of mixed infection of *Mycobacterium bovis* and *Mycobacterium avium* subspecies *paratuberculosis* was higher in cattle (11.1%) followed by goat (9.3%) and buffaloes (3.8%).

Conclusion: Present study reported the presence of mixed infection of bovine tuberculosis and paratuberculosis in domestic livestock species and raised renewed concerns regarding the zoonotic risk for humans of North India. Study indicated the need of surveillance and control programs for both the diseases to secure optimum animal production and human health in the country.
Active surveillance for human plague in Northwestern Uganda, 2008-2014

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Background: Plague is a life-threatening flea-borne zoonosis caused by *Yersinia pestis*. Over 95% of cases worldwide are reported from rural Africa. In Uganda, the active plague foci remains in West Nile region bordering Democratic Republic of Congo. To better define the epidemiology, clinical features, and outcome of human plague in Uganda, several surveillance strategies and networks used have significantly reduced morbidity and mortality.

Methods & Materials: Active surveillance, coupled with central laboratory support, was established in 10 clinics and 2 hospitals in the endemic West Nile region. Community awareness was enhanced though training of Village Health Team workers and a small number of traditional healers. For surveillance purposes, a suspect plague case was defined as rapid onset of fever and painful lymphadenopathy or hemoptysis. A confirmed case was defined as a suspect case with isolation of *Y. pestis* from a clinical specimen (blood, bubo aspirate, or sputum) confirmed by phage lysis, or a 4-fold increase in anti-F1 antibody titers. A probable case was defined as a suspect case epidemiological linked to a laboratory-confirmed case.

Results: A total of 256 cases were identified during January 2008 – March 2014, including 75 laboratory confirmed and 25 probable cases. The number of cases per full year ranged from a high of 153 in 2008 to a low of 6 in 2014. Median patient age was 11 years (range <1-70 yrs); 53% were female. Overall, 217 patients had bubonic, 20 septicemic, and 18 pneumonic forms of plague. Among 254 patients with a documented outcome, 26% (26/100) with confirmed or probable plague died, as compared with 8% (12/154) of those with suspect plague. Mortality among patients with pneumonic plague was 44% (8/18) overall, and 55% (5/9) for those with confirmed pneumonic plague. Antimicrobials used for treatment included doxycycline, gentamycin, chloramphenicol, co-trimoxazole, and ciprofloxacin. Forty-nine (19.6%) cases were outbreak-related.

Conclusion: Plague remains a public health concern in the West Nile region of Uganda. Although most cases are bubonic and sporadic, small outbreak of pneumonic plague with person-to-person transmission do occur, with the potential for larger outbreaks. Mechanisms to enhance prompt referral, rapid diagnosis, and effective antimicrobial treatment are needed to reduce mortality.
Comparison of serology, culture and polymerase chain reaction (PCR) for diagnosis of human brucellosis
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Background: Brucellosis is a zoonosis with worldwide distribution, especially in developing countries including Iran. Conventional methods for diagnosis of brucellosis include serologic tests and blood culture and for rapid detection of Brucella, PCR is available. The aim of this study was to compare serologic test, polymerase chain reaction (PCR) and BACTEC blood culture methods for detection of Brucella species in clinical specimens.

Methods & Materials: In a 15 months period, between 2013 to 2014, 149 patients with clinical suspected brucellosis were enrolled in this comparative-descriptive study. Clinical specimens were obtained for culture and serologic tests including wright, coombs' wright, and 2-Mercaptoethanol (2ME). Then Brucella DNA was extracted from blood specimens and PCR was performed using specific primers set. A questionnaire including demographic, clinical and paraclinical characteristics was completed for each subject.

Results: The most common symptoms were fever, malaise, myalgia and sweating. Osteoarticular complications were observed in 106 (71.1 %) patients, of which, the most common type was sacroiliitis in 67 patients (45%). Serologic tests including wright, coombs' wright, and 2ME were positive in 88.6%, 87.5% and 88.5%, respectively. Clinical specimen cultures were positive in 38.3%(n=57), that in 91.2% was positive, too. PCR were positive in 83 of 132 patients that 50 of these cases had positive culture that 100% of Brucella isolates were B.melitensis. There are significant correlation between PCR and culture. The sensitivity of PCR method was 96%, with specificity of 77%; the positive and negative serology predictive values were 100% and 78%, respectively.

Conclusion: Serologic tests are useful for the diagnosis of brucellosis in most cases. In patients with low titers of antibodies, blood and other clinical specimens' culture are helpful for definitive diagnosis. Those with negative culture and serology can be confirmed by PCR method. The PCR method is a valuable test for rapid diagnosis of brucellosis in clinical specimens.
Serological Survey and Identification of brucella spp of male breeding animals in Some Soums of Arkhangai Province, Mongolia

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Background: Aim of this study is to determine a prevalence of brucellosis of male breeding animals in some soums Arkhangai province and identify of brucella spp, circulating among animals.

Methods & Materials: For sampling were performed in two ways, for serological investigation were collected blood serum samples from all male-breeding animals, including bull, yak-bull, rums and billy-goats, with exception of stallion and male breeding-camels in these three soums, arkangai province and secondly, were collecte whole blood, swabs and other tissue for bacteriological gyteriology. In addition were collected milk and uterus swab for from aborted yak-cows and ewes. All sera were screened by RBT (IDVet, France) and positive samples were re-checked by iELISA (VLA, UK). For bacteriological examination was used a conventional methods to determine a proporties of isolates on grownt, clony and morphological characteristics and CO₂ requirement, urease, oxidase activity and etc. Genomic DNA of all brucella like isolates were checked by INgene Bruce-ladder V PCR kit (INGENASA, Madrid, Spain).

Results: A total of tested 925 bulls, 16.5% of 521 in Chuluut soum, 21.5% of 219 in Tsakhir and 7% of 185 in Tsetserleg were positive and total of tested 1948 rums and billy goats, 8.3% of 467 and 2.9% of 1145 were positive in Tsakhir and Tsetserleg soums, respectively. However, in aborted cows, 52.9% of 174 in Chuluut, 69.3% of 88 in Tsakhir and 15.4% of 13 in Tsetserleg soums were positive by RBT, respectively. All RBTest positive sera from aborted animals were re-tested by iELISA and 100% of samples in Chuluut and Tsetserleg soums, 98.4% in Tsakhir, 100% were positive. A 160 tissue samples were exemined bacteriologically. A total 30 of brucella-like organisms were isolated and out of these, 8 isolates have been confirmed as a B.abortus by classical bacteriological methods and PCR, respectively. Furtermore, laboratory examination is has been contiuining and isolates will be deterimmed at a biovar level.

Conclusion: This results suggested that, a prevalence of brucellosis among male breeding animals, including yak-bulls in above mentioned soums, Arkhangai province is comperatively high against average prevalence of disease in Mongolia.
Quantitative analysis of brucella spp in aborted bovine fetuses by real-time PCR
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Background: Brucellosis is a highly contagious zoonotic disease that has serious implications on human and animal health. In animals, brucellosis affects reproduction, causing abortion mainly in cows. The aim of this study was to determine the prevalence of brucellosis in different tissues of the bovine fetuses and to analyze whether the amount of the DNA detected changes after formaldehyde treatment and putrefaction.

Methods & Materials: The material studied was composed of 70 aborted cattle fetuses brought to Adana Veterinary Control and Research Institute during 3-year period. From each fetus, tissues from lung, liver, kidney, heart, spleen and abomasus were analyzed freshly, after 15 days of 10% formaldehyde treatment and after 15 days stay at 20 °C using commercial Brucella genus detection kit on Real-time PCR device (Roche Light Cycler 2.0).

Results: Brucella spp. was found positive in all of the tissues of 10 (14%) of the fetuses, lung revealing the highest DNA amount and both putrefication and formaldehyde treatment reduced significantly the DNA that could be detected by PCR, results after formaldehyde being better than putrefied material.

Conclusion: Real-time PCR is safe and sensitive technique for the detection of Brucella in tissues of abortus materials of infected animals.