Abstracts from the First International Science Symposium on HIV and Infectious Diseases (HIV SCIENCE 2012)
Chennai, India. 20-22 January 2012
Edited by Suniti Solomon, Sunil S Solomon, Pachamuthu Balakrishnan, Kailapuri G Murugavel, Shanmugam Saravanam, Hussain S Iqbal and Ramachandran Vignesh
Published: 4 May 2012
These abstracts are available online at http://www.biomedcentral.com/bmcinfectdis/supplements/12/S1

ORAL PRESENTATIONS

01 Engineering chemokines to develop putative anti-HIV-1 agents
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BMC Infectious Diseases 2012, 12(Suppl 1):O1

Background: Even though, Highly Active Antiretroviral Therapy (HAART) has resulted in significant reduction in mortality associated with Acquired Immune Deficiency Syndrome (AIDS), the side-effects and difficulties in patient compliance warrants the search for new therapeutic options. One potential strategy is to design chemokine analogues that will prevent the entry of human immunodeficiency virus-1 (HIV-1) into the target cell by competitively blocking its interaction with CC chemokine-5 (CCR5) receptor. The objective of the present study is to design and validate the efficacy of chemokine analogues based on the viral macrophage inflammatory protein-II (vMIP-II) core as putative anti-HIV agents.

Methods: In the present study we have synthesized the chemokine analogues by Fmoc solid phase peptide synthesis and were purified to homogeneity by semi preparative RP-HPLC. Molecular mass of the analogues were confirmed by MALDI-TOF MS. Structural characterization was done by CD spectroscopy. Their interaction with CCR5 receptor was analyzed by calcium release studies.

Results: The analogues designed were obtained in high purity and correct identity and they displayed similar patterns on the CD spectra to their parent template vMIP-II. The analogues displayed significantly lower toxicity in MTT assays. The interactive nature of the analogues was evident in calcium release studies where, the calcium flux varies with the type of the grafted N-terminus.

Conclusion: The findings in this study highlight the potential of vMIP-II to serve as a valuable target in designing chemokine analogues, which have the efficiency to serve as putative anti-HIV-1 agents.

02 Genotype-specific incidence and clearance rates of human papilloma virus (HPV) infection in HIV-infected women from Pune, India
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BMC Infectious Diseases 2012, 12(Suppl 1):O2

Background: Information on HPV genotype-specific incidence and clearance is important to better inform the natural history of cervical neoplasia in context of HIV/AIDS. We conducted a cohort study among HIV-infected women in Pune, India to estimate the genotype-specific HPV incidence and clearance rates.

Methods: HIV-infected women underwent baseline and annual follow-up visits for cervical cancer screening and collection of cervicovaginal samples for detection of 37 HPV genotypes by Linear Array polymerase chain reaction (PCR) assay.

Results: A total of 215 eligible participants were followed for a median time of 11 months (range: 8-23 months) with a follow-up period of 223 person-years. Of the 104/215 (48.4%) HIV-negative at baseline, 12 women were newly detected with HPV at follow-up visit reflecting an incidence rate of 5.4 per 100 person-years. Type-specific incidence rates ranged between 0.45-3.42 per 100 person-years for carcinogenic HPV types and between 0.45-1.79 per 100 person-years for other HPV types. Of the 111/215 (51.6%) women with HPV at baseline, 21 women cleared all types, reflecting a clearance rate of 9.4 per 100 person-years. Type-specific clearance rates ranged between 0.45-4.48 per 100 person-years for carcinogenic HPV types and between 0.45-4.04 for other HPV types.

Conclusions: This study adds to the scant global data of natural history of HPV infection in HIV-infected women. Knowledge of incidence and clearance rates can inform cost effectiveness and decision analysis models for estimating effectiveness of HPV vaccination and screening strategies for cervical cancer prevention for HIV-infected women.
03

**Immunodynamics of Th17 cells in HIV-1 subtype ‘C’ infection**

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**Background:** Th17 cells are IL-17 producing CD4+T cells which play a vital role in inflammatory responses, antimicrobial defense and autoimmunity. However, the involvement of Th17 cells in HIV-1 infection especially in subtype-C is not yet identified. Thus through this study we try to dissect the role of Th17 cells in HIV-1 subtype ‘C’ infection.

**Methods:** 31 HIV seropositive antiretroviral therapy naive and 8 HIV uninfected healthy control subjects were recruited and characterized as being early, late or slow progressor. Peripheral blood mononuclear cells were isolated from each study subject and stimulated with HIV-1 subtype ‘C’ gag peptide pool and assessed for IL-17 cytokine producing CD4+T cells using intracellular cytokine staining. All clinical groups were statistically compared by Kruskal-Wallis test and Spearman’s correlation coefficient was calculated for correlation of different variables.

**Results:** Here we reported that both frequency and functionality of HIV-1 specific Th17 cells were induced in early and slow progressors but were significantly reduced (p<0.001) at late stage of infection in peripheral blood. Also a significant negative correlation (p=0.55; P=0.0004) was observed between HIV-1 plasma viral load and gag specific IL-17 production via CD4+ T-cells.

**Conclusion:** This study showcases a comprehensive picture of Th17 cellular dynamics in HIV-1 subtype-C infection. Further, our data establishes that higher frequencies of HIV specific Th17 cells correlates with better control of viral replication and can be used as immune correlate of protection.

05

**Studies on HIV integrase-LEDGF/p75 interaction inhibitory activity of isatine derivative using the alpha screen luminescent proximity assay**

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**Background:** During the early stage of HIV-1 replication, integrase (IN) plays important roles at several steps, including reverse transcription, viral DNA nuclear import, targeting viral DNA to host chromatin and integration. Previous studies have demonstrated that HIV-1 IN interacts with a cellular lens epithelium-derived growth factor (LEDGF/p75) and that this viral/cellular interaction plays an important role for tethering HIV-1 preintegration complexes (PICs) to transcriptionally active units of host chromatin. Small molecule inhibitors of HIV IN/LEDGF have emerged as promising new class of antiviral agents for the treatment of HIV/AIDS. Present work is to study the small molecule inhibitor of HIV IN/LEDGF.

**Method:** Isatine-sulphadimidine derivative (SPIII-5H) selected for these studies. HIV IN/LEDGF interaction inhibition assay performed by ALPHA screen technique, HIV integrase assay investigated by oligonucleotide based assay and molecular modeling studies also carried out by using computational methods.

**Results:** Lead molecule SPIII-5H inhibits HIV IN/LEDGF interaction (protein-protein interaction) at 10 μM and HIV integrase activity at 6.8 μM. From molecular modeling study indicates that SPIII-5H bind with active site of HIV integrase (DDE), change the conformation and interrupt the binding of HIV integrase with LEDGF.

**Conclusion:** SPIII-5H novel class of inhibitors of HIV IN/LEDGF interaction and this lead molecule is suitable for further molecular modifications.

04

**Influence of CD38/PD1 co-expression on T cell subsets in HIV-TB co-infection**

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**Background:** Excessive immune activation as indicated by CD38 expression is a characteristic feature of HIV progression. There are growing evidences suggesting that immune inhibitory signals such as PD1 expression also play an important role in progression of the disease. However, the relationship between these positive (CD38) and negative (PD1) immune signals on T cells in HIV-TB co-infection has not been studied in detail so far.

**Methods:** Expression levels of CD4, CD8, CD38 and PD1 were analyzed in peripheral blood collected from HIV, TB, HIV-TB and healthy controls using standardized protocol on flow cytometer.

**Results:** The percentage CD8+/CD38+ and CD8+/PD1+ cells was high in HIV-TB and HIV when compared with control and TB, while there was no significant difference between HIV and HIV-TB. The CD38+/PD1+ co-expression was significantly high (p<0.01) on CD8+ cells in HIV-TB when compared with HIV, control and TB groups, inferring that CD38/PD1 phenotype distinguishes CD8 T-cell responses between HIV and HIV-TB co-infection.

**Conclusion:** High CD38+/PD1+ co-expression on CD8 cells probably causing a variation in CD8 cell responses could perhaps be a risk factor for development of tuberculosis in HIV-positive individuals. The use of CD8/CD38/PD1 markers for HIV-TB needs contemplation.

06

**Transcriptional modulation of HIV-1C LTR promoter**

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**Background:** All current anti-HIV1 therapies target the viral proteins or RNA; however targeting HIV1 at the transcriptional level of the integrated provirus has been less explored. In India, AIDS is commonly caused by HIV-1C compared to HIV-1B in developed countries. HIV1-5LTR acts as a promoter and shows sequence variation among different clades. Transcriptional gene silencing (TGS) is a method wherein dsRNA targeting the promoter/ enhancer of a gene are used to down regulate its expression.

**Methods:** We used SiHa cell line stably expressing a bi-cistronic reporter system (5’LTR-SEAP-IRES-EGFP), in which secreted alkaline phosphatase (SEAP) and enhanced green fluorescent protein (EGFP) are expressed under 5’LTR of HIV-1B/C. The cell line was transfected with different dsRNAs (S1-S6) targeting the core promoter/enhancer of HIV-1C LTR to induce TGS. Screening for decreased transcription was done using real-time PCR (mRNA expression of SEAP and EGFP), fluorescence microscopy (EGFP) and flow cytometry (EGFP).

**Results:** After single or multiple (thrice) transfection of dsRNAs, we identified one dsRNA (S4) which showed consistent and significant down regulation of both SEAP (44% & 68% respectively) and EGFP (40% & 65%) (p<0.001 in both cases) mRNA levels. This reporter down regulation was also confirmed by studying EGFP expression using fluorescence microscopy and flow cytometry which also showed a significant fall after S4 transfection.

**Conclusion:** TGS usually involves epigenetic modifications like DNA methylation/histone methylation at the targeted region and induces long term suppression of gene expression. So targeting of the HIV-1C LTR by dsRNA can be used as a therapeutic modality in the future.
O7
Analysis of drug resistance to HIV-1 protease using fitness function in genetic algorithm
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BMC Infectious Diseases 2012, 12(Suppl 1):O7

Motivation: Analysing the potential organic molecule for inhibiting HIV-1 protease against its drug resistance by predicting its fitness using Genetic Algorithm will enhance research in the discovery of identifying the potential lead for inhibiting the aspartyl protease of type IV L.

Methods: Drug resistance is predicted for all FDA approved HIV-1 protease inhibitors and organic leads synthesized by Dr. Deeb and Dr. Godzari with wild type and mutant strains of subtype B. Initially the structural feature of HIV-1 protease with the inhibitor complex has been analysed on the basis of "Binding Energies". Finally the fitness function in Genetic Algorithm was used for optimizing the inhibition of specific organic lead with three fold cross validation.

Results: Structural data mining performed by the fitness function in Genetic Algorithm gave pattern identities between HIV-1 protease (wild type and mutants) of sub type B against organic leads and FDA approved inhibitors of HIV-1 protease. Genetic Algorithm gives >80% Accuracy for wild type inhibition and >75% Accuracy for mutant inhibition in the final optimization by fitness function.

Conclusion: Organic leads have greater affinity than the FDA approved inhibitors (specifically Mol-23 which has good correlation with pIC50 and H Bonding descriptors). I84V mutant inhibitors and organic leads synthesized by Dr. Deeb and Dr. Godzari with wild type and mutant proteases of HIV type I.

O8
Characterization of gene family that mediates the adhesion of biofilms formed by Candida tropicalis isolated from HIV and non-HIV patients
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BMC Infectious Diseases 2012, 12(Suppl 1):O8

Background: Candida tropicalis is an important cause of candidemia in immunocompromised patients. Biofilm formation helps the organism to establish infection. Agglutinin like sequence (ALS) genes encodes glycoproteins which are important adhesins. This study was done to detect the presence of ALS genes by PCR in C.tropicalis strains isolated from HIV and non-HIV patients in comparison with biofilm formation.

Materials and methods: Yeast isolates: A total of 48 C.tropicalis isolates (HIV-20; non-HIV-28) were included in this study. Biofilms were formed on 96 well plates as described earlier and ALS genes were detected by PCR using specific primers.

Results: Among the 48 C.tropicalis isolates, 16 out of 20 (80%) HIV isolates and 17 out of 28 (61%) non-HIV isolates were biofilm producers; 4 out of 20 HIV isolates (20%) and 11 out of 28 (39%) non HIV isolates were biofilm non-producers. Out of 48 isolates, 12/48 (25%) isolates were positive for ALS 1; 24/48 (50%) isolates were positive for ALS 2; 23/48 (48%) isolates were positive for ALS 3. Thirty four out of 48 (71%) isolates were positive for one or more ALS genes. Twenty two of the 34 (65%) were biofilm producers. Of the 14 strains which were negative for all ALS genes, 11 (79%) were biofilm producers.

Conclusion: ALS 2 and ALS 3 genes were more common in C.tropicalis than ALS 1. The biofilm forming ability of the strains was independent of the presence of the ALS genes and the source of the isolates.

O9
Spectrum of adverse cutaneous eruptions to nevirapine – a cross sectional study
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Background: HIV infection increases the risk of adverse drug eruptions. The reason for this is unclear; the mechanism probably involves drug specific cytotoxic lymphocytes. The aim of the study was to observe the spectrum of adverse cutaneous eruptions to nevirapine.

Methods: PLHIV initiated on nevirapine based ART regimens between 2010 and 2011, were included in the cross sectional study. These hospitalized patients were followed up for the sequence of events. A detailed concomitant drug history was taken to rule out other impending drugs which can cause similar drug reactions.

Results: In this analysis, 80% were females, 70% heterosexuals, 20% homosexuals and 10% intravenous drug users. Average age of patients was 31.70 ± 9.89 years. The spectrum of drug eruptions ranged from erythematous maculopapular generalized rash in 20% of patients to grade four SJS & TEN in 80%. Regression and inverse growth model was employed to find out the significant duration between the onset of nevirapine rash and initiation of ART. Mean duration of onset of nevirapine rash was 12.40 ± 3.78 days, with mean CD4 count at the time of drug reaction was 162.10 ± 32.61 microns/dL. It was observed that the correlation co-efficient (r=0.981) between the onset of nevirapine rash and CD4 count was significant (P<0.05), which clearly depicts the association of the onset of nevirapine rash to low CD4 count (<200 microns/dL).

Conclusion: NVP rash can show polymorphic manifestations, and the majority of them occurs within first 4-6 weeks of initiation of ART and is often associated with low baseline CD4 count.

O10
Detection of norovirus in stool samples by RT-PCR in 5 disease centers in Iran
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Background: Noroviruses are a group of viruses that cause gastroenteritis (illness that usually includes diarrhea and/or vomiting) in people. Gastroenteritis means inflammation of the stomach and small and large intestines. Many different viruses can cause gastroenteritis, including rotaviruses; noroviruses; adenoviruses, types 40 and 41; sapoviruses; and astroviruses. Current techniques used for detection of noroviruses in stool samples include multi-step viral RNA extraction and purification followed by reverse transcriptase-polymerase chain reaction (RT-PCR). The aim of this study is the detection of norovirus in stool samples by RT-PCR in 5 disease centers in Iran.

Methods: In this descriptive study, 2170 stool samples of patients consulting for acute gastroenteritis at a pediatric hospital in 5 cities of Iran were enrolled. The mean age of the study population was 48 months with an age range of 30 days to 4 years. Fecal specimens were collected within 24hrs of admission. The specimens were frozen, sent to the laboratory, and then stored at -80°C until being tested for norovirus.

Results: RT-PCR was evaluated with 2170 stool samples containing 90 (4.14%) norovirus-positive (0.97% Tehran, 0.64% Tabriz, 0.18% Mashhad, 1.57% Shiraz, 0.78% Bandar Abbas). The RT-PCR was validated with published primers for norovirus (JV12/JV13). In both retrospective and prospective settings, the RT-PCR was equally sensitive and specific in detecting norovirus.
Conclusion: Acute gastroenteritis can be caused by norovirus. It has to be attended to vaccination against norovirus after rotavirus.

O11 Renal manifestations and associated factors among HIV infected children at Muhimbili National Hospital, Dar es Salaam, Tanzania
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BMC Infectious Diseases 2012, 12(Suppl 1):O11

Background: Human Immunodeficiency Virus infection is a global challenge and sub-Saharan African countries contribute significantly to this pandemic. Children are vulnerable and acquire the infection mostly from their mothers. Highly Active Anti-retroviral Therapy have led to dramatic changes in the incidence of opportunistic infections with reduction in morbidity and mortality, which has paved way for manifestation of non-infectious complications including renal complications. The aim of this study was to determine prevalence of microalbuminuria, proteinuria and associated factors among Tanzanian children.

Methods: We recruited 240 HIV infected children attending care and treatment clinic. Microalbuminuria and proteinuria were determined by using dipstick and Microalbumin 2-1 combo test strips on spot urine respectively. Serum Creatinine, white blood cell and CD4 counts were determined. Renal ultrasound examinations were also performed.

Results: Forty nine children (20.4%) had microalbuminuria and 17 (7.1%) had proteinuria. Prevalence of proteinuria was significantly higher among children aged 120 months and above (p-value<0.05). Lower CD4 percent (<25%) was a risk factor for microalbuminuria (p-value<0.01) and proteinuria (p<0.01). Mean CD4 count was significantly lower in children with microalbuminuria (p-value<0.05) and proteinuria (p-value<0.001). Twenty eight (11.7%) children out of 153 had increased cortical echogenicity on ultrasound examination.

Conclusion: Proteinuria and increased cortical echogenicity were prevalent among HIV infected children who may indicate early onset of renal complications and this call for routine screening for early detection.

O12 Porcine endogenous retroviruses: an obstacle to cross xenotransplantation
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BMC Infectious Diseases 2012, 12(Suppl 1):O12

Background: Xenotransplantation involves the transplantation of non-human tissue or organs to humans. Worldwide shortage of organs for clinical applications has shifted the focus towards non-human sources. Pig represents a rich source of organ donors but the presence of porcine endogenous retroviruses (PERVs) represents a particular risk and considered as a major obstacle during xenotransplantation. Among various types; PERV-C may recombine to form recombinant PERV-A/C and has the ability to infect human cells invitro and replicate at high titers. Our study aims to screen porcine tissue samples for provirus and virus particles of PERVs by PCR and reverse transcriptase PCR (RT-PCR).

Methods: A total of 23 porcine heart tissue samples were included in this study. DNA and RNA from tissue samples were extracted using DNA and RNA extraction kits respectively. All the samples were subjected to standard PCR to detect pro-viral DNA and RT-PCR for mRNA expression of virus particle using specific primers.

Results: All the 23 (100%) samples tested were positive for PERV-A and B by RT-PCR. Fifteen out of 23 (65.2%) and 5/23 (21.7%) samples were positive for PERV-C and PERV-A/C pro-viral DNA respectively. Five out of 23 (21.7%) and 3/23 (13%) samples were positive for PERV-C and PERV-A/C RNA respectively.

Conclusion: Low prevalence of PERV-C in our study indicates and paves a way to cross the obstacle in xenotransplantation by using PERV-C free animal which may not produce infectious PERV-A/C recombinant virus.

O13 PVL positive methicillin resistant Staphylococcus aureus breast abscess infection among post-partum women in Chennai, South India
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BMC Infectious Diseases 2012, 12(Suppl 1):O13

Background: Breast abscess and mastitis caused by MRSA is on the rise. This could result in poor response to routinely used β-lactam antibiotics. Lack of MRSA screening could lead to wrong choice of antibiotic after drainage resulting in multiple and recurrent abscesses leading to increased morbidity and mortality.

Methods: A total of 20 isolates of Staphylococcus aureus were isolated from 25 cases of breast abscess from a tertiary hospital during January 2010-October 2010. Isolation was done by culturing the pus sample drained from the abscess with 16 gauge needle. The S. aureus isolates were screened for susceptibility to various antibiotics including cefoxitin, clindamycin, fusidic acid, mupirocin, vancomycin and linezolid. MIC to mupirocin, clindamycin and fusidic acid were performed using E-strip method for resistant isolates. Molecular detection of methicillin resistance and Panton-Valentine Leukocidin (PVL) were done by multiplex PCR targeting femA, mecA, and lukSP-PV.

Results: Of the 20 isolates, 10 (50%) were found to be MRSA, of which 3 (30%) isolates were found to be positive for PVL. Of the tested isolates, highest sensitivity (100%) was observed for vancomycin, linezolid, mupirocin, clindamycin and fusidic acid. Highest resistance was observed for co-trimoxazole-erythromycin-inofloxacin netilmicin-gentamicin-amikacin. High-level methicillin resistance (MIC = 256µg/mL) was observed for 3 MRSA isolates from hospitalized patients.

Conclusion: From the above results, the PVL-MRSA infections in breast abscess among post partum women is found to be increasing. Proper drainage and routine screening of MRSA from breast abscess should be done to decrease the morbidity and mortality.

O14 Segmentation of sputum smear images for detection of tuberculosis bacilli
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Background: Tuberculosis (TB) is a common and lethal infectious disease, which requires accurate and early diagnosis for effective containment. Essential for the diagnosis of pulmonary infection is the detection of the bacilli through the manual microscopic examination ofZN-stained sputum smear, which is a time-consuming, complex process necessitating at least 8-10 minutes per slide. Moreover, the quality of the detection is highly subjective to the individual who performs the analysis. These results can clearly be improved upon by using image processing techniques. The proposed work uses the segmentation techniques to automate the analysis of the sputum smear images and to detect the presence of tuberculosis bacilli in them.

Methods: This study involves assessing ZN-stained images obtained using a digital camera DFC280 attached to a compound microscope. The images acquired are 24-bit coloured tiff images. The software was...
developed using MATLAB R2009b. It has been deduced from the images, that the bacilli have a distinct colour and shape. The aim of the study is to identify the rod-shaped coloured bacilli, which are 1-10μm long. Colour based segmentation and property information, combined with morphological operations are used for bacilli detection.

Results: A prototype application was developed to identify these bacilli. The sensitivity, specificity, PPV and NPV of the study is 68.75%, 93.75%, 91.67% and 75% respectively.

Conclusions: The above study shows the potential in harnessing image analysis techniques for the detection and study of the TB bacilli. Texture analysis and identifying overlapping bacilli are required for more accurate results.

O15 HLA-A*0201-specific epitopes of Indian HIV-1C as candidates for vaccine design
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Background: HLA alleles are associated with differential outcomes of infections/diseases. We hypothesize that epitopes that interact with HLA alleles associated with resistance elicit a protective immune response in the host, and could therefore serve as good vaccine candidates. Among the HLA alleles reported to be associated with resistance to HIV infection/slow progression to AIDS, HLA-A*0201 occurs most frequently in the Indian population. We undertook this study to identify HLA epitopes specific to this HLA allele from HIV-1C.

Methods: 1769 sequences of all proteins of Indian HIV-1C were downloaded from the HIV sequence database and consensus sequence for clade sequences isolated from India using insilico approach. The short-listed epitopes could be tested for their potential as vaccine candidates for the Indian HIV epidemic. This approach has been extended to identify epitopes specific to other HLA alleles associated with resistance to HIV, and in vitro evaluation is being undertaken.

O16 The global distribution of CCR5 delta 32 polymorphism: role in HIV-1 protection
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Background: The human immunodeficiency virus type 1 (HIV-1) infection occurs by binding to CD4+ receptor and chemokine receptor 5 (CCR5) or the CXCR chemokine receptor (CXCR4). A mutation in the CCR5 gene, 32 base pair deletion consequences a truncated protein that is not expressed on the cell surface. The deletion confers resistance to HIV-1 infection and slows the progression of AIDS in HIV infected individuals. The worldwide distribution of CCR5delta32 polymorphism was congregated by retrieving the data from literature and genotyping new population samples. A comprehensive resource of frequency data for CCR5delta32 polymorphism in different populations samples was created.

Methods: The data for different populations was obtained from literature. In order to investigate the genetic variation in CCR5 gene in new population, we analyzed 257 healthy control individuals. We examined the CCR5 32 base pair deletion (CCR5-Δ32) by conventional polymerase chain reaction (PCR).

Results: The genotype frequency distribution of CCR5 in new population was found to be (CCRS / CCR5: 98%, CCRS / CCR5-Δ32: 2% and CCR5-Δ32 / CCR5-Δ32: 0%) in healthy control.

Conclusion: The allele frequency of CCR5-Δ32 observed for new population is 1% which is compared with the other populations. The polymorphism CCR5-Δ32 is primarily found in European population. Compared to our data, frequency of delta 32 deletion is observed at high frequency in European populations. Our data of delta 32 deletion is significantly different from Caucasians (p<0.000000001), Africans (p<0.01458) and Europeans (p<0.000000001).

O17 Ethnic differences in efavirenz CNS toxicity – role of cytochrome P450 2B6 polymorphisms
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BMC Infectious Diseases 2012, 12(Suppl 1):O17

Background: Highly active antiretroviral therapy regimens that include efavirenz are effective, but adverse events are common, especially central nervous system (CNS) toxicities. We previously showed increased discontinuation rates of efavirenz due to CNS toxicities in Malay and Chinese patients in our Treat Asia HIV Observational Database (TAHOD) database. We postulate that these differences may be due to genetic differences.

Methods: We performed genome wide genotyping with the Illumina 1M and the Affymetrix 6.0 microarrays in healthy volunteers from the 3 major ethnic groups in Singapore, consisting of Chinese (mainly of southern Chinese origin), Malays and Indians (mainly of southern Indian origin). Genetic information was available from 95 Chinese, 89 Malays and 82 Indians.

Results: Allele frequencies were obtained for the rs3745274 single nucleotide polymorphism coding for the cytochrome P450 2B6 (CYP2B6) 516 G>T mutations in Singapore. The frequencies of these mutations are significantly higher than in Caucasians (p<0.000000001). The frequencies of these mutations are 22.1% in Chinese, 37.6% in Malays and 37.8% in Indians. Homozygous TT mutations were found in 2.4% in Chinese, 12.4% in Malays and 12.2% in Indians. This homozygous TT mutation frequency is higher than that found in the European American population, which was reported to be 3%.

Conclusion: Our study showed significant ethnic differences in CYP2B6 516 G>T mutations in Singapore. The frequencies of these mutations are significantly higher than in Caucasian populations. This finding may explain the higher discontinuation rate of efavirenz due to CNS side effects. Further studies with studying the association of CYP2B6 genotype with CNS toxicities and efavirenz concentrations are indicated.

O18 Molecular determinants analysis and co receptor tropism prediction of V3 loop of HIV-1 “C” clade sequences isolated from India using insilico approach
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BMC Infectious Diseases 2012, 12(Suppl 1):O18

Background: The V3 loop of the env gene in HIV-C type is considered as a major viral determinant for coreceptor specificity. The tip motif of V3 loop is known for antibody neutralization, so sequence variation in this motif have an impact on virus infectivity and disease progression. So analysis of genetic diversity in the V3 loop Tip motif help us to improve the CCR5 antagonist’s development as well as vaccine target.
Methods: HIV-1 "C" type env sequences of Indian isolates were retrieved from HIV sequence database. Coreceptor Tropism prediction by different web-based interpretation system like (WebPSSM, Geno2pheno, (ds) Kernel, WetCat) was performed. The diversity of V3 loop tip motifs, glycosylation motifs were analyzed using N-Glycosite. Relative frequencies of each amino acid in V3 loop were determined using Web Logo.

Results: In this study, lower numbers of positive charges of v3 loop, in the range of 4 to 5, reveals the prevalence of RS tropism. Thus majority of the V3 sequences of HIV-1 Indian isolates were predicted as RS-tropism and we identified few mutational prevalence like for RS-predicted viruses -E2SD, Y21F and for K4-predicted viruses E25Q, F12, H34Y. Overall eight different tetrameric tip motifs (GPgQ, GPgR, GPQR, GPpR, gPQQ, EPgQ, and GgSQ) were identified.

Conclusion: High prevalence of RS tropism, higher no of conserved motif regions in the V3 sequence among HIV strains in India reveals the need of potentialCCR5 antagonists. GPgQ and GPgR is highly conserved in all the sequences and this pattern could be an ideal target for AIDS therapy.

O19 Neutralization of tier-2 viruses and epitope profiling of plasma antibodies from subtype-C HIV-1 infected north Indians; implications for MPER directed HIV-1 neutralization

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BMC Infectious Diseases 2012, 12(Suppl 1):C19

Background: Evaluation of the broad and potent neutralizing antibody (bNAb) responses in sera of HIV-1 infected individuals is important for immunogen design.

Methods: Plasma of eighty naïve HIV-1 seropositive Indian patients were tested for neutralization against a panel of 3 subtype-B and 5 subtype-C tier 1 and tier 2 viruses. Three plasma, found to be broadly neutralizing (bNP), were mapped with a set of 212 consensus-C gp160 overlapping peptides (15mer each). 50% binding titers were determined by ELISA using consensus-C V3 (35mer), IDR loop (19mer) and MPER (24mer) peptides.

Results: The patients (30 females and 50 males; age range: 20-57 years) had been infected for different time periods (few days to seven years). The viral load ranged from 156-2180000 RNA copies/ml, mean CD4 count was 346 cells/mm³ while mean total IgG levels was 12.3 mg/ml. 64 plasma samples and HIV-1 viral load were evaluated for high risk of progressive primary TB.

Plasma of eighty naïve HIV-1 seropositive Indian patients were screened for CCR5-directed neutralization. 80% of the plasma samples showed neutralization against 4 subtype-B and 3 subtype-C HIV-1 viruses. 16% of the plasma samples showed neutralization against 5 subtype-B and 2 subtype-C HIV-1 viruses. The neutralization titers were compared with the neutralization titers of MPER and IDR peptides. Overall, eight different tetrameric tip motifs (GPgQ, GPgR, GPpQ, GPpR, gPQQ, EPgQ, and GgSQ) were identified.

Conclusion: High prevalence of RS tropism, higher no of conserved motif regions in the V3 sequence among HIV strains in India reveals the need of potential CCR5 antagonists. GPgQ and GPgR is highly conserved in all the sequences and this pattern could be an ideal target for AIDS therapy.

O21 Anti-malarial chloroquine metamorphosed into antiviral agent against HIV with four modes of actions

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BMC Infectious Diseases 2012, 12(Suppl 1):O21

Background: To identify an economical and effective antiviral agent we are nurturing chloroquine (CQ) which is an antiviral agent with multimodal and immune modulatory actions. Earlier studies have reported inhibition of HIV by CQ through its anti-integrase activity and also had elucidated that CQ inhibits HIV by inhibiting glycosylation of envelope glycoprotein. It had also been shown that CQ blocked Tumor-Necrosis-Factor (TNF-) alpha and Interleukin-6(IL-6): production and interferes with HIV replication.

Method: In vitro assay of anti HIV action of CQ in MT4-cells and clinical studies.

Results: We observed Inhibitory Concentration (IC50) 7.19µg/ml and Cytotoxic-Concentration (CC50) >35.43 and the maximum protection noted was 90%; a laudable observation. Results on hand; we launched a preliminary study of giving CQ at 2 tables per day as add-on drug along with other antiretroviral agents to 6 HIV patients proved by ELISA and Western blot; age ranged between 6 and 48. We have observed CD4 and CD8 cells count improved by 20% to 35% but their sustaining effects couldn’t be assessed because two patients died due to overwhelming intercurrent infection and four patients had good improvement for 2-3 yr and lost for further follow up. This report of ours is next to first Indian report.

Conclusion: We could ascertain that this add-on drug CQ had been tolerated well and major side effects were not observed and with good anti HIV action.

O22 HIV-TB the deadly duo, the biggest health challenge in Fiji

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BMC Infectious Diseases 2012, 12(Suppl 1):O22

Background: Tuberculosis is a common opportunistic infection and HIV patients with latent TB infection are at risk of reactivation and those with recently acquired infection are at high risk of progressive primary TB. Approximately 1000 people in the western pacific region die from the
disease every day. The threat of increasing HIV rates fueling the TB epidemic has become an important massive challenge in Fiji to the control of TB at all levels. Hence this study was to evaluate the rate of TB co-infection among HIV patients in Fiji.

Methods: This study involved the retrospective descriptive analysis of the data available in the P1 Twomey Hospital, Suva, Fiji. All registered cases of HIV and HIV-TB has been included in the study.

Results: By 2010, Fiji has 191 registered cases of tuberculosis and 393 HIV positive. The % of TB patients with known HIV status is 100 because of the recent Fiji HIV/TB surveillance policy which recommends that HIV testing is mandatory in all newly diagnosed cases of TB for all health care settings. Fiji has reported 2% of new TB patients every year which are also HIV positive and the mortality rate of HIV/TB patients is very high (80%).

Conclusion: The HIV positive co-infected with TB than patients who are HIV negative. Hence the TB/HIV co-infected were started TB therapy prior to ARVs. TB treatment reduces the burden of HIV in people living with TB.

POSTER PRESENTATIONS

P1

Co-infectivity of hepatitis B virus and hepatitis E virus
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BMC Infectious Diseases 2012, 12(Suppl 1):P1

Background: Viral hepatitis is a major health problem and is an important cause of morbidity and mortality throughout the world. Present study is aimed to assess the co-infection of hepatitis E virus with hepatitis B virus in HBV-DNA positive cases.

Methods: A total number of 40 adult patients from various liver diseases with high level of ALT and AST were included in this study and were analyzed for hepatitis viral marker including HBsAg and IgM anti-HEV. The detection of HEV-RNA in 32 HBV-DNA positive cases was done by Real Time PCR.

Results: The results of this study demonstrate that hepatitis E virus (HEV-RNA) infection to be rare (6.25%) in HBV-DNA positive cases. Surface antigen (HBsAg) for hepatitis B virus was positive in 18 cases (45%) and IgM Anti HEV was positive in 16 cases (40%). Hepatitis B virus infections, as the predominant causes of liver diseases, HBV-DNA was detected in 32 cases out of 40 cases and HEV-RNA was detected in 2 cases out of 32 HBV-DNA positive cases respectively. Co-infection of both HBV and HEV were reported in acute and chronic liver diseases in more than 18% cases by ELISA and 6.25% cases by real time PCR.

Conclusion: HBV and HEV are the major cause of acute and chronic liver diseases. The data from this study indicates that presence of HEV-RNA in HBV-DNA positive cases, is rare in population. The co-infection of hepatitis B virus and hepatitis E virus in various liver diseases may occur.

P2

Opportunistic protozoa in HIV seropositive cases and best stool concentration technique for detection
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BMC Infectious Diseases 2012, 12(Suppl 1):P2

Introduction: Parasitic gastrointestinal diseases increase morbidity and mortality in HIV patients. This study is aimed at the occurrence of Cryptosporidium, Isospora, Cyclospora and Microsporidium in the stool samples of HIV positive cases since the diarrhea is the second most common presentation of HIV positive cases who requires hospitalization.

Materials and methods: Stool specimen from HIV infected patients (n=100) were included. Each time specimens were divided into two portions of which one was plain and second part mixed with 10% buffered formalin saline in 3:1 ratio. Blood samples were collected for lymphocyte counts. Samples were processed and compared with Formal-Ether sedimentation and Sheather's sugar floatation technique for the detection of oocysts.

Results: Isospora belli was predominant opportunistic protozoa detected. Cryptosporidium oocysts were found in 2 cases of acute diarrhea and one case with chronic diarrhea. No Cyclospora and Microsporada were detected. Sheather's sugar floatation technique is found better in concentrating the oocysts of Isospora and Cryptosporidium. Along the oocysts, 2 cases of Angiostrongylus duodenale, one case of each Giradia lamblia and Strongyloides stercoralis were detected.

Conclusion: While testing for detection of protozoan parasites from HIV cases, it needs to collect multiple stool samples if feasible. Sheather's sugar floatation technique is superior to Formal-Ether sedimentation to detect the oocysts of Cryptosporidium and Isospora belli. Absolute lymphocyte count is probably good when used as a marker for CD4 count assessment where the facilities are not available for CD4 count testing.

P3

In-silico designing of acyclic nucleoside phosphonates and their anti-HIV potential
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BMC Infectious Diseases 2012, 12(Suppl 1):P3

Background: Reverse transcriptase, the viral enzyme, is a key target in the search for effective drugs useful for AIDS therapy and has critical roles in the life cycle of the human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS. HIV replication can be blocked by inhibition of the enzyme HIV RT.

Method: The object of our study is to develop newer nucleoside phosphonates analogs bearing unsaturation and modifications in heterogenous bases and prediction of their anti-HIV potential. Designing is done keeping in the Lipinski’s Rule of Five in focus. The diphosphates of compounds have been docked into the active site of wild type HIV-RT (PDB: ID 2B6A). The forcefield of the Chemistry at Harvard Macromolecular mechanics (CHARMM) was applied to 3D models of PD HIV RT-nevirapine complex and synthesized ligands. The energy function is based on separable internal coordinate terms and pair wise non-bond interaction terms.

Result: Docking studies revealed that the diphosphates of acyclic phosphonates had good interactions with various amino acid residues present in the active site of HIV RT. The Ludi 3 score was found to be 718 and the corresponding Kd value was 0.06µM. This is in good agreement with the observed value of 0.05µM.

Conclusion: On the basis of SAR studies, uridine phosphonate analogs are expected to be probable lead molecules against HIV-RT.

P4

Study of pulmonary involvement in HIV-seropositive patients
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BMC Infectious Diseases 2012, 12(Suppl 1):P4

Background: HIV causes diseases of respiratory system in various ways. The lung involvement may be of infective etiology or non-infective like Kaposi’s sarcoma or pulmonary hypertension. In India with the load of pulmonary tuberculosis, immuno-compromised patients are exposed to the risk of tuberculosis. This study endeavors to identify the patients who get lung involvement secondary to HIV infection and impact of CD4 cell count on the severity of tuberculosis in HIV positive patients.

Methodology: Observational cross sectional study of 50 HIV seropositive patients with pulmonary involvement attending OPD and admitted in Pad Dr D Y Patil Medical College and Research Centre, Mumbai.

Results: 50 HIV patients were studied for 2 years. Majority of patients were in the age group of 40-49 years. 68% were males and 32% females. 62% patients had pulmonary TB. 28% had pleural effusion, PCP infection 2%, and pulmonary hypertension in 8%. Infiltrative lesions (42%) were more...
common X-ray findings. Sputum AFB positivity was seen in 52%. Mean CD4 count being 163.7 cells/µl. Most patients (70%) had CD4 count <200 cells/µl. Mean CD4 counts in patients with sputum positive TB was 183.8 cells/µl, in extra pulmonary tuberculosis was 174.9 cells/µl, in sputum negative TB was 93.8 cells/µl and in milary TB it was 77.7 cells/µl.

Conclusion: There can be variety of lung involvement in HIV positive patients who can have many pulmonary infections as well as pulmonary hypertension, which may be a major cause of morbidity and mortality in HIV positive patients. CD4 counts were very low in military TB and sputum negative pulmonary TB.

P5
Polyester dendrimers: a versatile nanocarrier for target specific drug delivery
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BMC Infectious Diseases 2012, 12(Suppl 1):P5

Background: Improving the therapeutic index of drugs is a major impetus for innovation in many therapeutic areas such as HIV, cancer, inflammatory and other infectious diseases. Polyester based dendrimers constitute a very attractive class of materials because they are less toxic, biodegradable and biocompatible. Our aim is to develop a novel ester based versatile dendritic nanocarrier that could specifically deliver drugs against HIV, cancer and other infectious diseases.

Methods: The monomer molecule was chemically synthesized and characterized for the development of nanocarrier. The poly ester based dendrimeric nanocarrier was synthesized by using solid phase organic synthesis. The multifunctional nanocarrier was characterized by UV, IR, NMR, MALDI-TOF, optical scattering and TEM. In-vitro toxicity studies, bioavailability and biocompatibility studies were carried out using specific receptor over expressing cell lines.

Results: Novel core-shell type polymer with surface specific amino functional groups was used for the nanocarrier synthesis. The solid phase Michael addition reaction was carried out using double ester acrylate monomer with amino group of the linker. The dendrimeric nanocarrier was allowed to develop up to the 3rd generation. The drug molecule was attached to the surface of the carrier using enzyme cleavable tetrapeptide spacer. The targeting ligands were attached on the surface of the nanocarrier for in-vitro studies.

Conclusion: This novel dendrimeric nanocarrier with multiple functional sites has tremendous potential as drug delivery vehicle. It could revolutionize the field of chemotherapy and the delivery of toxic, insoluble anti HIV, anticancer and peptide based drugs of short half life with reduced cytotoxicity.

P6
Evidence for the roles of oxidative stress, nitrative stress and NF-KB activation in Tenofivir Disoproxil Fumarate (TDF) induced renal damage in rats
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BMC Infectious Diseases 2012, 12(Suppl 1):P6

Background: Nephrotoxicity is a dose limiting side effect of Tenofivir (TDF), a commonly used anti-HIV drug. About 37-45% of patients on anti-HIV drugs suffer from renal damage. As the mechanism of pathogenesis of TDF induced renal damage is not clear, it is necessary to elucidate the mechanism in order to prevent the renal damage. This study investigates the roles of nitrative-oxidative stress and NFκB activation in TDF induced renal damage.

Methods: Rats were administered 2 daily doses of TDF (300 mg/kg body weight) by gavage for 35 consecutive days, control rats received water alone. On the 35th day, the rats were sacrificed, and the kidneys were used for histological examination, immunohistochemical analysis, and assay of the activities of antioxidant enzymes, myeloperoxidase and NFκB. Data were analyzed with Mann Whitney U test.

Results: TDF administration to the rats resulted in renal damage. Electron microscopically, damage to the mitochondria of the proximal tubules was observed. Statistically significant increase in protein carbonyl content and nitrate levels (p<0.008), decrease in reduced glutathione (61%, p<0.01), protein thiol (33%, p<0.03), and activities of the antioxidant enzymes was observed. A 16 fold increase in the activity of NFκB (p<0.05), and a 9 fold increase in myeloperoxidase activity (p<0.01) were observed in the kidneys of TDF treated rats. The renal tissues of TDF treated rats stained strongly for nitrotyrosine and PARP.

Conclusion: Nitro-oxidative stress and NFκB activation contribute to TDF induced renal damage in rats. The source of these free radicals may be the damaged mitochondria and activated neutrophils.

P7
Development of Efavirenz nanoparticle for enhanced efficiency of anti-retroviral therapy against HIV and AIDS
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BMC Infectious Diseases 2012, 12(Suppl 1):P7

Background: The FDA approved drug Efavirenz is a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) successful first line drug of choice in Highly Active Anti-Retroviral Therapy (HAART) for treatment of HIV and AIDS. It is poorly water soluble drug (10 g/ml) with 40-45% bioavailability and administered as high doses 600-800 mg/day. Increase in solubility can enhance bioavailability; providing reduction of dose, resistance and harmful side effects.

Methods: Efavirenz nanoparticles are developed using methacrylate polymers (Eudragit E100) by emulsion solvent evaporation method (1:0.5, 1:1, 1:2 and 2:1 ratios) and the in-vitro evaluations such as particle size, morphology, solubility changes, drug release, compatibility and cytotoxicity tests are carried out.

Results: The particle size of 99-200 nm with narrow size distribution and surface charge (-52 V) shows high stability. The formulation with entrapment efficiency (75-90%) shows higher drug release profile 95-100% within 1 hour compared to 23%-58% of pure drug in water, 0.1N HCl and phosphate buffer pH 7.4 media. The DSC, TG-DSC, powder XRD and SEM morphology results reveal that there is solid transition from crystalline structure to amorphous state, which supports the solubility enhancement. The FT-IR gives the compatibility results for drug with other excipients. The Efavirenz nanoparticles subjected for in-vitro cytotoxicity and cell uptake studies using monocytes / macrophages (THP-1) proved better uptake (Floccytometry and Confocal microscope) of nanoparticles than free drug.

Conclusion: The solubility enhancement due to nanozising helps in hastening the drug release and also increasing cell uptake, which helps in attaining high bioavailability with low dose of Efavirenz.

P8
Characterization of natural HIV-1 Tat and Vpr variants from Northern India
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BMC Infectious Diseases 2012, 12(Suppl 1):P8

Background: HIV-1 Tat & Vpr are multifunctional and involved in transactivation, cell cycle regulation, MHC-1 modulation etc. Like other HIV genes, Tat and Vpr are subject to variation. Recombination frequency is higher in the first exon of Tat and Vpr. Characterization of these variants is the subject of the present study.

Methods: HIV-1 Tat exon 1 and Vpr were amplified from the DNA isolated from blood of HIV infected patients and cloned. Clones were sequenced and aligned against reference sequences using CLUSTAL W. Sim plot
analysis was done for recombinants. Their expression was accessed by transfection of HEK 293T cells with myc fusion clones of variants and western blotting using anti-myc antibody. The variant Tat clones were co-transfected with LTR-luc to investigate their LTR transactivation potential by dual luciferase reporter assay.

**Results:** Exon 1 of Tat was amplified from 21 samples and Vpr from 16 samples. Four Tat exon 1 and two Vpr sequences were found to have unique variations. Among the four unique Tat variants, one resembled subtypes B and C. This recombination in Tat was found to negatively affect its transactivation potential of reference strain B LTR in comparison with native Tat. Two Vpr sequences resembled subtypes B, C, and D at different locations. One variant had a frameshift towards C-terminus.

**Conclusion:** Variations in Tat affect the functional aspects of the protein including interactions with other viral proteins with consequences for virus-host interaction.

**P9**

**Development of a vaccine delivery system using hepatitis B core antigen based VLPs to deliver mycobacterial antigens**

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**BMC Infectious Diseases 2012, 12(Suppl 1):P9**

**Background:** Growing prevalence of TB and the emergence of XDR-TB have stimulated substantial efforts to develop better vaccines for TB. Recent researches have shown that some of the antigenic proteins and fusion of different proteins produced by *Mycobacterium tuberculosis* can give protection in animal models when administered with specific adjuvants. In the present study, we explored the use HBcag-VLPs for delivery of *tuberculosis* antigens.

**Methods:** HBcVLVPs bearing ESAT-6 and CFP-10 were constructed using PCR and recombinant DNA methods. Proteins were expressed in E. coli and purified. VLVPs formation was confirmed with TEM. BALB/c mice were immunized with VLVPs and controls without any adjuvants. Sera were analysed for antibody responses (ELISA). Splenocytes were cultured and restimulated with purified antigens and CF (culture filtrate) of M.tb. The cell proliferation was measured using cell proliferation assay kit and the culture supernatants were analysed for IL-2, IFN-γ and TNF.

**Results:** The recombinant VLP induces preferentially a Th1-type immune response against mycobacterial antigen even though Th2 has been reported as the predominant response in BALB/c mice. IFN-γ, IL-2, TNF and proliferation were significantly higher in mice immunised with HBcVLVPs-M. tuberculosis antigen. Restimulation with mycobacterial CF also produced the same effect.

**Conclusion:** The humoral and cellular responses suggest that the VLP containing fusion constructs generated immune response in a Th1 dependent manner. By virtue of its self-adjuvant nature, HBc VLVPs are a better vaccine delivery system for use with newer antigens identified in the course of recent developments in subunit protein vaccine research in *tuberculosis*.

**P10**

**In vitro anti-HIV activity of crude extracts from Tinospora cordifolia**

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**BMC Infectious Diseases 2012, 12(Suppl 1):P10**

**Background:** Human immunodeficiency virus (HIV) infection causes acquired immune deficiency syndrome (AIDS) and is a global public health emergency. Anti-HIV therapy involving chemical drugs has improved the life quality of HIV/AIDS patients. However, emergence of HIV drug resistance, side effects and the necessity for long-term anti-HIV treatment are the main reasons for failure of anti-HIV therapy. Therefore, it is essential to isolate novel anti-HIV therapeutics from natural resources. The aim of the present study was to evaluate the invitro anti-HIV activity of *T. cordifolia* plant extracts.

**Methods:** Extracts were prepared from dried leaves in n-hexane, dichloromethane, ethyl acetate and n-butanol. A toxicity study was performed on all crude extracts using peripheral mononuclear blood cells (PBMCs) isolated from whole blood. HIV-1 RT inhibition activity of the all solvent extracts of *T. cardifolia* was determined using a commercial kit.

**Results:** Among the tested extracts, the n-hexane and n-butanol crude extracts of *T. cordifolia* showed moderate cytotoxic activities against PBMCs with CC50 values ranging from 5.7–12.0 µg/ml. In the HIV-1 reverse transcriptase assay *T. cordifolia* plant extracts showed good inhibitory activity, which was near that of the reference drug. Ethyl acetate extract shows 85 percentage of HIV-1 RT inhibition activity at a concentration of 20 µg/ml.

**Conclusion:** The leaves of *T. cardifolia* extracts are shows anti-HIV 1 activity and this plant has great potential for developing useful drugs. Extraction of important biologically-active phytochemicals from this plant will certainly be helpful in protecting and treating various viral diseases in human beings.

**P11**

**Generation and characterization of human monoclonal single chain variable fragments (scFvs) against envelope third variable region (V3) of HIV-1 clade C**

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**BMC Infectious Diseases 2012, 12(Suppl 1):P11**

**Background:** Production of human monoclonal antibodies with broad neutralizing activity is an essential part of HIV-1 prophylactic vaccine. Majority of the viruses infecting Indian patients belong to clade C. Methods: A phage library of 7000 clones was constructed from a drug naive HIV-1 clade C infected Indian patient whose plasma exhibited high potential neutralizing potential against a panel of viruses and also displayed cross-reactive anti-V3 antibodies. PBMCs were isolated and EBV transformed. Cells (wells) producing anti-V3 antibodies were preselected with V3-CTB fusion protein and expanded. Total RNA was isolated and cDNA was constructed followed by VH and VL amplification. scFvs were constructed, cloned into phagemid vector and expressed in Escherichia coli. We assessed the expression of the scFvs by SDS-PAGE and Western blotting. Specificity was examined by ELISA. 10 clones were randomely selected after biopanning and checked for their binding to V3 peptides of clade C and B. Ten clones showed binding in phage ELISA, 8 were cross-reactive to both the V3 peptides while the other 2 were specific to V3C. The clones did not show cross-reactivity against other unrelated peptides. The recombinant anti-V3 scFvs (32kD) were expressed and confirmed by SDS-PAGE and Western blotting. DNA fingerprinting analysis showed that 9 out of the 10 clones were distinct.

**Conclusion:** This is the first report on the generation of human anti-V3 scFvs against HIV-1 clade C. Further assessment of the neutralization efficiency of these scFvs would reveal their potential for passive immunotherapy.

**P12**

**Association of GT microsatellite polymorphism in TLR 2 gene with leprosy**

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**BMC Infectious Diseases 2012, 12(Suppl 1):P12**

**Background:** Toll-like receptor 2 (TLR2) is critical in bringing up immune responses to mycobacterial infections. The mutations in TLR2 are known to confer susceptibility for severe infection with mycobacteria. TLR2 may diminish response to mycobacterial proteins and place individuals at risk
of developing leprosy. We investigated the association of GT repeat polymorphism in intron2 of TLR2 gene with leprosy in south Indian patients.

**Methods:** A total of 20 leprosy patients and 45 contacts were enrolled in the study. Primers were designed using Primer3 software for PCR amplification of the TLR2 gene. The number of GT repeats was confirmed by sequencing. Two-tailed Chi-Square test was performed to check the association. p less than 0.05 was considered to be statistically significant.

**Results:** The number of GT repeats varied from 13 to 24 in both the groups studied. The frequency of patients with (GT) 13 repeats was significantly low (p=0.04, OR=9.318) and that of (GT) 14 repeats (p=0.04, OR=7.76) was significantly high.

**Conclusion:** Our results suggest that an individual with (GT) 13 repeats may be resistant and those with (GT) 14 repeats may be susceptible to leprosy. Furthermore, elucidation of functional relevance studies such as gene expression and proteomics may reveal the influence and role of these repeat number variations in leprosy.

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**P13**

**Effect of nanoencapsulation on the phenomenon of drug interaction between anti-M. avium drug, rifabutin and anti-HIV drug, ritonavir by employing poly (lactide-co-glycolide) nanoparticles**

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BMC Infectious Diseases 2012, 12(Suppl 1) P13

**Background:** Interaction between M. avium and HIV drugs is unavoidable in HIV patients as for an improved life expectancy of HIV patients, additional medications have to be administered along with HIV drugs. The anti-M. avium drug rifabutin and protease inhibitor, ritonavir are associated with significant drug interactions involving the cytochrome P450 (CYP) enzyme system. Little information is available on the role of various drug delivery strategies in amelioration of interactions between anti-M. avium and anti-HIV drugs. The purpose of the present study was to evaluate effect of encapsulation of rifabutin and ritonavir in PLGA nanoparticles on the already known drug interaction exhibited by these drugs.

**Methods:** This study was designed including administration of rifabutin and ritonavir singly and in combination in free and nanoencapsulated form to swiss albino mice. Blood samples were taken following drug administration at various time intervals and pharmacokinetic parameters were assessed as: area under plasma drug concentration over time curve (AUC0–∞), mean residence time (MRT) and Cmax etc.

**Results:** Overall, nanoencapsulation was observed to avoid the known adverse drug interactions between RBT and RTV in the drug interaction study. (p-value<0.001).

**Conclusion:** Our results clearly emphasize the potential of the PLGA nanoparticle formulation to minimize drug interactions as the encapsulated drugs did not result in any significant alteration in kinetic parameters upon co-administration. It can be reasonably stated that nano-encapsulation would not only permit intermittent dosing but also a more favorable pharmacokinetics which further can overcome the drug interactions.

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**P14**

**Evaluation of anti-viral activity of Jatropha curcas leaf extracts against potentially drug-resistant HIV isolates**

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BMC Infectious Diseases 2012, 12(Suppl 1) P14

**Background:** Drug-resistant HIV, a major global concern, warrants the development of novel anti-virals as alternative and inexpensive therapy. In the current study, we isolated potentially drug-resistant HIV and assessed previously unreported anti-viral activity of Jatropha curcas leaf extracts.

**Methods:** *In vitro* micro-co-culture was employed for virus isolation followed by drug susceptibility assays to determine resistance to Azidothymidine (AZT) and Lamivudine (3TC).

Jatropha curcas leaves were extracted using Soxhlet apparatus. Methanolic (ME) and aqueous (AE) extracts were chosen for further study. Secondary metabolites were detected by High-Performance Thin Layer Chromatography and *in vitro* cytoxicity established by MTT assay. Anti-viral activity was evaluated by p24 inhibition in post- and pre-infection interaction studies.

**Results:** Seven HIV isolates were obtained (isolation rate: 23.33%) with drug IC50 values ranging from 0.001418-82.73 μM AZT and 2.645-13.5 μM 3TC.

Tannins, flavonoids, saponins were detected in AE and flavonoids, saponins in ME while CC50 values were 32.07 mg/mL AE and 35.5 mg/mL ME.

In post-infection studies (4 isolates), IC50 values were ranging from 0.0255-0.4137 mg/mL AE and 0.00073-0.1278 mg/mL ME; pre-infection studies (1 isolate) showed 100% p24 inhibition by ME and 97.19% p24 inhibition by AE at 25 mg/mL each.

**Conclusion:** HIV isolates potentially resistant to AZT/3TC were obtained; genotypic drug resistance was ascertained. Jatropha curcas leaf extracts showed effective anti-viral and probable entry inhibition activity against potentially drug-resistant HIV, which has not been reported earlier. We conclude that *Jatropha curcas* a good candidate for anti-HIV therapy with further research.

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**P15**

**Role of Mycobacterium avium catalase-peroxidase (KatG) in the pathogenesis of MAC disease in HIV patients**

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BMC Infectious Diseases 2012, 12(Suppl 1) P15

**Background:** The mycobacterial catalase-peroxidases protect it from the reactive oxidative metabolites and allow its survival within the host phagocytes. We compared the virulence of *Mycobacterium avium* complex (MAC) clinical isolates with varying catalase activity recovered from the blood of HIV patients in terms of invasiveness and intracellular multiplicity in host cells. The catalase activity in MAC isolates was also analysed in context to CD4 counts and clinical presentation of mycobacterial disease in HIV patients.

**Methods:** Catalase activity of KatG protein of 51 mycobacterial isolates from HIV patients was determined. MAC isolate with maximum catalase activity (KatG-max) was compared to isolate having minimum activity (KatG-min) for adherence, intracellular replication and katG mRNA expression by ZN staining, colony forming units (CFU) enumeration and RT-PCR respectively in A549 and HT29 cell lines.

**Results:** Catalase activity of mycobacterial isolates was found to be inversely related to CD4 counts and unrelated to the clinical presentation of mycobacterial disease in HIV patients. The intracellular replication of KatG-max isolate was found to be 2 fold higher than KatG-min at 3rd day of infection (do) [p<0.001], whereas, it was comparable at 1st do. CFU enumeration results correlated well with the levels of katGm RNA expression.

**Conclusion:** The MAC isolates having maximum catalase activity and increased katGm RNA expression were favoured for its survival and replication in the host cells. High levels of catalase activity in isolates from HIV patients with low CD4 counts suggest an important role of KatG in the establishment and progression of disseminated MAC disease.

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**P16**

**Familial study revealed the association of Vitamin D receptor gene haplotype with Hansen’s disease**

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BMC Infectious Diseases 2012, 12(Suppl 1) P16
**Background:** Single-nucleotide polymorphism within the gene encoding Vitamin D receptor (VDR) – a member of the nuclear receptor supergene family, is associated with several infectious diseases. The receptor belongs to the family of trans-acting transcriptional regulatory factors. Studies on VDR gene polymorphism reveals Fok I, Taq I, & Apa I restriction site variants to be significantly associated with many of the diseases compared to other SNPs within the gene. The study aims to determine the association of these polymorphisms with Hansen’s disease.

**Methods:** The study group included six well defined multilocus leprosy families with cases (n=32) and unaffected family members (n=44). Genotyping was done for the polymorphic positions present in exon 2(T/C), 9T/C and intron 8(C/A) regions of the VDR gene using Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism using enzymes Fok I, Taq I & Apa I respectively. Haplootype analysis was performed for the three positions using Chi square test in SNPSTAT software.

**Results:** Out of all possible combinations on haplotype analysis, C-T-C (p=0.018) and T-T-C (p=0.028) was negatively associated with Hansen’s disease and no significant association was observed with individual gene variants. The wild alleles at position Taq I and Apa I were found to be in strong linkage disequilibrium.

**Conclusion:** The data indicates that a relationship exists between VDR polymorphic haplotype and the development of disease and the haplotypes C-T-C and T-T-C may perhaps render protection against Hansen’s disease.

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**P17**

Rapid and inexpensive drug susceptibility testing for Mycobacterium tuberculosis by a nitrate reductase assay (NRA) from western India

**Introduction:** Increase in multi-drug resistant (MDR) Mycobacterium tuberculosis (MTB) strains is a worrisome trend seen during recent years. Rapid detection of MDR strains is very important to restrict their spread in the population. Gold standard methods for drug susceptibility testing (DST) of MTB are either costly or very slow. Nitrate reductase assay (NRA) is one of the methods for rapid detection of resistance. This technique is based on the capacity of M. tuberculosis to reduce nitrate to nitrite. The WHO recommends that the NRA be used as direct test on smear-positive sputum specimen or as an indirect test on Mycobacterium tuberculosis isolates grown from conventional solid cultures. We evaluated the performance of NRA as rapid, reliable & inexpensive method for drug-susceptibility testing of Mycobacterium tuberculosis against first line antitubercular drugs, Rifampicin (RIF) and Isoniazid (INH).

**Methods:** 80 strains of M. tuberculosis isolated from sputum samples of pulmonary tuberculosis patients were subjected to NRA and absolute concentration method for comparison.

**Results:** Out of 80 isolates, 12 strains were resistant to INH & 11 strains were resistant to RIF and 9 strains are resistant to both INH & RIF constituting MDR strains. Sensitivities and specificities were 99%, 98% for RIF and 99%, and 100% for INH by NRA as compared to Absolute Concentration. However median time of obtaining results was shorter using NRA (9-10 days) compared to Absolute Concentration (30-40 days).

**Conclusion:** We conclude that NRA has the potential to be a useful tool for rapid DST of Mycobacterium tuberculosis.

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**P18**

The study of hepatitis B surface antigen and anti-HCV in HIV infected patients

**Background:** HIV is known to influence the natural history of infections with HBV and HCV and interactions between HIV and these hepatitis viruses may potentiate HIV replication. The present study deals with the co-infection of HBV and HCV in HIV infected patients and also with the mode of transmission of HBV and / or HCV in HIV infected patients with special reference to high risk groups.

**Methods:** The study group included 106 HIV positive patients with history of heterosexual contact and the control group included 30 asymptomatic healthy individuals. The sera of patients were tested for HBsAg and anti-HCV using third generation ELISA kits. The samples positive for first kit were retested for confirmation of results. The sera of control group were simultaneously screened for HIV, HBsAg, and anti-HCV by ELISA method.

**Results:** In the study of 106 HIV seropositive patients, 22 (20.75%) were positive for HBsAg and 5 (4.72%) were positive for anti-HCV. One patient (0.94%) was positive for both HBsAg and anti-HCV. Among the 30 healthy controls, one individual (3.3%) was positive for HBsAg and none were positive for HIV and anti-HCV. The co-infection of HIV and HBV in the study population was statistically significant when compared to controls (p<0.005).

**Conclusions:** The sexual transmission of both HBV and HCV is of epidemiological importance in the light of heterosexual transmission of HIV in India. Monitoring of HIV infected patients for concurrent infection with HBV and HCV is therefore necessary.

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**P19**

A study on speciation and antifungal susceptibility pattern of Candida isolates from HIV patients with oropharyngeal candidiasis and correlation with CD4 count

**Background:** Oral candidiasis is a clinical predictor for progression to AIDS. Antifungal drug resistance is becoming a major problem with this immunedefeated populace. Considering the above facts, the study was conducted to speciate and to determine the susceptibility pattern of the Candida isolates from HIV patients with oral candidiasis and to correlate it with the CD4 count of the patients.

**Materials and methods:** Fungal isolates collected from (n=150) the lesion using sterile cotton swabs in HIV patients with oral candidiasis. Isolation and speciation were done by standard mycological procedures. Antifungal susceptibility was determined by Microbroth dilution method, as per the CLSI guidelines. Estimation of CD4+ T lymphocyte of the patients was done by FACs count system.

**Results:** Of the 150 samples, two revealed a mixture accounting for the 152 isolates. Candida albicans 118 (78%) was the most common species followed by Candida tropicalis 17 (11%), Candida krusei 8 (5%), Candida parapsilosis 6 (4%), Candida glabrata 2 (1%) and Candida guilliermondii 1 (1%). By microbroth dilution 18 (11.8%) isolates were fluconazole resistant, 23 (15.1%) were itraconazole resistant and all were amphotericin susceptible. Of the 150 patients, 106 (70.6%) had CD4 count <200 cells/ μl. Azole resistant was more common in patients with CD4 count <200 cells/ μl.

**Conclusion:** Candida albicans is the most frequently isolated species. Non-albicans Candida species are emerging as important pathogens with increasing rates of azole resistance and with increased immunosuppression. This emphasizes the need for speciation and determination of susceptibility pattern of the Candida isolates from HIV patients with oropharyngeal candidiasis.
Background: In 1986, a second type of HIV, called HIV-2, was isolated from AIDS patients in West Africa, where it may have become present decades earlier. HIV-2 infections are predominantly found in Africa. West African nations report a prevalence of HIV-2 infection of more than 1% in the general population. The prevalence rate of HIV-2 infection in India is not available so far as HIV-2 infections are underreported in the country. The current use of Rapid Antibody diagnostic tests when used singly are unable to specifically detect HIV-2 and HIV 1&2 infections. Hence the present study was undertaken to specifically detect HIV-2 and HIV 1&2 infections and validate the currently used Rapid test kits using HIV specific peptide EIA and Western Blot test. A total of 37 serum samples (18 HIV-2 and 19 HIV 1&2) were evaluated by using HIV specific peptide EIA (CDC Atlanta, USA) and Western Blot test. All these 37 samples were diagnosed HIV-2 and HIV 1&2 as per the guidelines laid down by the National AIDS Control Organization (NACO), India.

Results: 100% concordance was observed in the results of Rapid test, EIA test and Western Blot test.

Conclusion: Under all circumstances screening for diagnosis of HIV should be based on three different tests with three different antigenic principles. Using above protocol it is possible to detect HIV-2 and HIV 1&2 infections.

P21 Incidence of bla genes among uropathogenic Escherichia coli isolates from HIV and non-HIV patients in South India
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BMC Infectious Diseases 2012, 12(Suppl 1):P21

Background: Group 3a/b cephalosporins are currently being used in the treatment of UTI and urosepsis. However, Extended Spectrum Beta-Lactamase (ESBL) mediated resistance has been increasingly reported among uropathogens from HIV patients. We sought to determine the incidence of ESBL genes- bla CTXM, bla TEM, and bla SHV among E. coli isolates from HIV (with increased exposure to cephalosporins) and non-HIV antenatal patients.

Methods: PCR detection of bla CTXM, bla TEM and bla SHV were carried out among ESBL positive E. coli isolates from HIV (n=57) and non-HIV antenatal patients (n=22). Fisher’s exact test was employed to analyze the statistical significance of the results.

Results: Overall, 31.7%, 59.5% of the E. coli isolates carried bla TEM, bla CTXM respectively, while none harboured bla SHV. When stratified based on host group, significant difference was observed in the incidence of bla CTXM among the isolates from HIV and non-HIV patients (70.2% vs 31.8% respectively, p = 0.0024; OR 5.03; 95% CI = 1.74-14.57). Nonetheless, difference in prevalence of bla TEM among the HIV and non-HIV isolates was not statistically significant (29.8% vs 36.4%, p = 0.5979). Co-occurrence of bla TEM and bla CTXM was detected among 22.8%, 0% of the E. coli isolates from HIV and non-HIV patients respectively (OR 5.1477; 95% CI = 1.3766-19.2273).

Conclusion: Our results augment the fact that frequent exposure to cephalosporins serves as the driving selection force leading to increased incidence of ESBL (bla CTXM) mediated resistance among the E. coli isolates from HIV patients. Hence, the risk associated with antimicrobial exposure needs to be considered in therapeutic decision making.

P22 Occurrence of Porphyromonas gingivalis fimA type II and prtC genotype among periodontitis patients
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BMC Infectious Diseases 2012, 12(Suppl 1):P22

Background: Porphyromonas gingivalis fimbriae are classified into six genotypes (types I-V and Ib). Among them, occurrence of fimA type II genotype is more predominant in periodontitis patients. Similarly collagenase encoded by prtC gene is a potential virulence factor expressed by P. gingivalis strains associated with periodontal disease. The study was opted to detect the presence of P. gingivalis fimA type II and prtC genotypes in periodontitis patients.

Methods: Subgingival plaque samples collected from 128 chronic periodontitis (ChP) and 72 aggressive periodontitis (AgP) patients were subjected to PCR to screen for the presence of fimA type II and prtC gene of P. gingivalis. Chi-square test was employed to compare the prevalence of the genotypes.

Results: The prevalence of P. gingivalis fimA type II genotype among ChP, AgP and health was 50.5%, 45.3% and 13.60%, respectively. Similarly, prevalence of P. gingivalis prtC genotype among ChP, AgP and health was 49.5%, 45.3% and 9.10% respectively. P. gingivalis type fimA+/prtC+ genotype were present in 28.9% of ChP, 33.3% of AgP patients and 4.5% of healthy subjects. Patients positive for both the genes showed probing depth of ≥7mm. Significant difference was observed between periodontitis and healthy subjects for all the three genotypes (P<0.001).

Conclusion: The results show that P. gingivalis fimA type II and prtC genotypes are equally associated with chronic and aggressive periodontitis. The predominance of P. gingivalis fimA type II + prtC+ genotype in teeth with deep pockets or serious attachment loss, suggest their role in periodontal destruction.

P23 Enhanced frequency of neutrophils and inflammatory monocytes and diminished numbers of T and B cells in active pulmonary tuberculosis
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BMC Infectious Diseases 2012, 12(Suppl 1):P23

Background: Mycobacterium tuberculosis (M.tb) infects nearly 2 billion people worldwide. Effective immunity against M.tb infection requires co-ordinated responses from both innate and adaptive arms of immunity. To elucidate the immune responses important both in control of infection and in extra-pulmonary dissemination, we examined frequency and/or absolute numbers of T, B and NK cells, dendritic cells and other leucocyte populations in active tuberculosis patients.

Methods: The frequency as well as absolute numbers of T cells (CD3+, CD4+, CD8+ T cells), B cells and NK cells as well as the frequency of innate immune cells (neutrophils and monocytes), dendritic cell subsets (pDC & mDC ), T cell subsets (naive, central and effector memory and regulatory T cells) was examined by flow cytometry in AFB smear positive pulmonary TB (Sm+) (n=30) and AFB smear negative pulmonary TB (Sm-) (n=24) and compared with extra-pulmonary TB (EP) (n=38).

Results: Among the innate immune subsets, we observed significantly higher frequency of neutrophils and inflammatory monocytes in Sm+ pulmonary TB group when compared with Sm- pulmonary and EP TB group. On the other hand, the absolute numbers of CD3+ T cells, CD4+ T cells, CD8+ T cells and B cells were significantly lower in Sm+ when compared with Sm- and EP TB group.

Conclusion: Pulmonary TB is characterized by enhanced frequencies of neutrophils and inflammatory monocytes and diminished absolute counts of T and B cells, suggesting a crucial role for these cell populations in protection against TB disease development as well as extra-pulmonary dissemination.

P24 Cross clade reactive plasma anti-V3 antibodies in human immunodeficiency virus type-1 infected individuals develop with time
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BMC Infectious Diseases 2012, 12(Suppl 1):P24
Introduction: Timely transfusion of blood saves millions of lives but unsafe transfusion practices puts many people at risk of transfusion transmissible infection (TTI). TTI can exist as an asymptomatic disease in the host; so, donors must be screened for high risk behaviour related diseases.

Aim: To estimate the seroprevalence of HIV, HBsAg and HCV among whole blood donors.

Methodology: This study was conducted at Department of Transfusion Medicine, Sri Ramachandra University, Porur, Chennai during July-2010 to Jun-2011. The sample size in this study was 11,871. All samples were subjected to ELISA screening for anti-HIV 1 & 2, HBsAg and anti-HCV in addition to other mandatory tests.

Results: Out of 11,871 samples, seroprevalence of HIV, HBsAg and HCV were estimated to be 0.05%, 1.58% and 0.13% respectively during the study period.

Conclusion: Stringent screening of donors for TTI is crucial to ensure safe supply of blood and blood products. This study involved both voluntary and replacement donors. Prevalence of TTI was found to be low in females. Highly sensitive ELISA kits play a major role in detecting antibody to HIV, HCV and HBsAg. Furthermore, Nucleic Acid Testing (NAT) facilitates viral detection at a much earlier stage and therefore reduces the chances of window period infection transmission to 1 in a million.

P25
A prospective analysis of viral immune escape in the chronic phase of the subtype C HIV-1 infections of India
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BMC Infectious Diseases 2012, 12(Suppl 1):P25

Background: HIV-1 is capable of evading CTL immune response through mutations in residues both within the epitopes and in sequences flanking the epitopes leading to viral diversity - a major obstacle for vaccine design. The present study aims at identifying the HLA-restricted CTL escape mutations primarily in the asymptomatic phase, and examining a correlation between such mutations and disease progression.

Methods: In a prospective study, a cohort of select seropositive drug-naive subjects categorized into one of three clinical groups (long-term non-progressors, regular progressors and rapid progressors), based on the archived clinical records at YRG CARE, are being monitored for two years with repeated sampling at 6-month intervals. The phylogenetic analysis of the gag sequences was inferred using the Neighbour-Joining method in MEGA 5 to monitor viral evolution and to study CTL escape. ELISPOT will be used for mapping of the immunodominant CTL epitopes in gag with an emphasis on identifying a possible correlation between such immune responses and disease progression.

Results: Twenty plasmid clones of gag have been sequenced from ten patients each at the base level. Escape mutants in several known immunodominant CTL epitopes have been identified in many of the subjects. The viral isolates phylogenetically clustered with the reference subtype C sequences. Additionally, multiple sequences from individual viral isolates clustered together indicating genetic-relatedness. Sequence analysis with REGA HIV-1 Subtyping Tool further confirmed the subtype C identity of all the viral isolates.

Conclusion: The preliminary data are suggestive of viral escape in the chronic phase of the viral infection.

P26
Seroprevalence of transfusion transmissible viral infections among blood donors in a tertiary care hospital
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BMC Infectious Diseases 2012, 12(Suppl 1):P26

Background: Subtype-C alone accounts for approximately 50% of global and more than 95% human immunodeficiency virus-1 (HIV-1) infection in India. Identification of antigenic epitopes that induce antibodies with cross-clade activity will be crucial to address the HIV-1 viral diversity.

Methods: 80 HIV-1 infected drug naive patients were recruited for this study. The study was approved by the institute ethics committee and informed consent was obtained from all the participants. The relative binding of anti-V3 polyclonal plasma antibodies to 35 mer consensus-B and C V3 peptides was done by ELISA binding assay. Statistical analysis was performed by GraphPad Prism 5.

Results: Assessment of the relative binding revealed that 86% (69/80) and 99% (79/80) of the plasma were able to reach an IC50 binding titer with V3B peptide and V3C peptides respectively; with substantially low antibody titer that bind to V3B than V3C (mean IC50, V3B=2736 versus V3C=12612) (p<0.0001). The finding suggests that although majority of the antibodies were subtype specific, a good proportion of cross reactive anti-V3 antibodies also exist in these plasma (mean=23% range=0.11-97%). We observed a positive correlation between percent cross reactive anti-V3 antibodies and days from first diagnosis (p=0.008) while no such association was found with other clinical and immunological parameters like plasma viral load (p=0.24), CD4 count (p=0.34) and total plasma IgG levels (p=0.45).

Conclusions: This is the first study to demonstrate the presence of cross-clade reactive anti-V3 antibodies and their association with time in the plasma of HIV-1 infected Asian Indians from north India.
Background: Acinetobacter are gram negative, catalase positive, oxidase negative, nonmotile, non fermenting cocobacilli. The emergence of Acinetobacter infection is uncommon in organ systems that have a high fluid content. The nosocomial infections including, respiratory tract, CSF, peritoneal fluid, urinary tract, and endotracheal aspires (ET) are the place where they have the capabilities to accumulate and cause in-hospital and in-community infections. The rate of emergence of Acinetobacter spp with multi drug resistance property is increasing in different geographical regions. Due to the properties of MDR the recent treatments of such infections has become difficult. In the present study evaluation of emergence of MDR nonfermentative Acinetobacter spp was done from the niche of organ system of patients with high fluid content.

Method: The nonfermentative Acinetobacter spp were isolated from different clinical samples according to standard procedures and Gilardi schemes. The antibioticotyping of Acinetobacter was done by disk diffusion method as per CLSI standards.

Results: 31.12% (62/193) of nonfermentative Acinetobacter spp were isolated from different clinical samples. The ratio of resistance against different antibiotics among Female:Male was found to be 1:1.38. All Acinetobacter spp were multi-resistant and showed different multi drug resistance pattern. Acinetobacter spp from samples showed varied resistance including, 60% resistant strain from wound swabs, 50% from Pus, 28-30% from ET aspirates and blood. Conclusion: Acinetobacter spp poses significant problem worldwide and increasingly responsible for numerous infections. Our study shows the emergence of high rate of MDR of Acinetobacter spp against 18 antibiotics belonging to different groups.

P29 Prevalence of multidrug resistant tuberculosis at tertiary care hospital
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Background: Tuberculosis continues to plague the world and remains the major global health problem. Simultaneously the incidence of drug resistant Mycobacterium tuberculosis strains is also increasing in almost all industrialized and developing countries.

Methods: This prospective study was done at NMC, Nellore from July 2008-December 2009. Samples received at microbiology lab for acid fast staining were included in this study. Smears were stained by Ziehl-Neelsen’s technique. Samples were cultured on Lowenstein-Jensen media after processing by modified Petroff’s method and incubated according to CLSI guidelines. Identification of Mycobacterium tuberculosis was done based on morphology, nitrate reduction test and catalase test. Drug susceptibility for first line anti-tubercular drugs was performed by proportion ratio method.

Results: A total of 2031 samples were included in this study. 120 samples were smear positive by acid fast staining, 110 were culture positive for Mycobacterium tuberculosis. 16 (14.5%) samples were resistant to one or more antitubercular drugs. 10 (9.09%) samples showed monodrug resistance, Isoniazid (3.63%) followed by Rifampicin (2.72%) Ethambutol (1.81%) and Streptomycin (0.90%). Isoniazid and Ethambutol resistance in one sample (0.90%). Isoniazid and Rifampicin resistance in two samples (1.88%). Three samples were resistant to Isoniazid and Rifampicin along with other drugs (2.72%). HIV co-infection among MDR-TB was 2.7%.

Conclusion: According to the present study prevalence of MDR-TB was 4.54%. Among the patients on treatment higher incidence of resistance was attributed to poor patient compliance in spite of effective DOTs programme.

P30 Prevalence of HAV and HEV in the patients presenting with acute viral hepatitis
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BMC Infectious Diseases 2012, 12(Suppl 1):P30

Background: Hepatitis A and E are both enterically transmitted resulting in sporadic and epidemic forms of acute hepatitis in developing countries including India. This study was done to determine the prevalence of HAV and HEV in the patients presenting with acute viral hepatitis and also to determine co-infection of HAV and HEV in these patients.

Methods: The cross-sectional study was conducted in Kasturba Medical College, Manglore. A non-random sampling of 958 patients presenting with acute viral hepatitis were included. On the basis of history, serum samples were analyzed for IgM anti HAV and IgM anti HEV for the detection of hepatitis A (HAV) and hepatitis E (HEV) respectively using commercially available ELISA kits.

Results: The seroprevalence of HAV and HEV positive patients were 19.31% and 10.54% respectively. The prevalence of both HAV and HEV in patients with acute viral hepatitis was 11.5%. The prevalence of HAV and HEV among males (68% & 31%) was higher than in females (31% & 20%). HAV and HEV were predominantly seen among young adults. These infections were predominantly seen during later part of rainy and beginning of winter season.

Conclusion: Though the prevalence of HAV is much higher than that of HEV, co infection rate of 11.5% mandates the screening for HEV which will be of immense importance in pregnant women and improving levels of personal hygiene among higher socio-economic population. These data will be essential for planning of future vaccination strategies and for better sanitation programme in this part of the country.

P31 Serological and molecular diagnosis of hepatitis B virus
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BMC Infectious Diseases 2012, 12(Suppl 1):P31

Background: Hepatitis B is a potentially life threatening liver infection which leads to millions of deaths annually. Serological and molecular assays for hepatitis B virus are the major diagnostic tools. Dried blood spot (DBS), a minimally invasive procedure, is an alternative to serum and can be used for field based studies for molecular detection. The present aim of this study is to diagnose HBV infection by combination of serological and molecular methods from serum and dried blood spot.

Methods: Blood was collected from suspected cases of liver diseases attending JIPMER Hospital, during September 2010 to October 2011. The study group is divided into two each having 30 cases of hepatitis B surface marker positive and negative profile respectively. Samples were analyzed for complete serological tests (surface and core antigen) and PCR were performed on serum samples and DBS.

Results: Out of 30 HBsAg positive cases screened by ELISA, 22 samples were found positive of HBV DNA by PCR method from serum, which includes 2 samples with only surface (HBsAg) and antibody to core antigen (IgM anti HBC) positive. All these 22 positive cases were also been detected from DBS after storing the sample at 25°C for 4 and 7 days.

Conclusion: It is important to detect both serological and molecular markers to diagnose hepatitis B for appropriate management of disease. Since dried blood spot yields comparable results to serum in detecting HBV DNA it can be used as convenient method of collecting samples than venous blood, particularly in resource limited settings.

P32 Rapid diagnosis of extra pulmonary tuberculosis by automated Xpert MTB/RIF assay
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BMC Infectious Diseases 2012, 12(Suppl 1):P32

Introduction: Although extra pulmonary tuberculosis accounts for only 10-15% of tuberculosis in India, they have high morbidity and mortality because of lack of good diagnostic methods. One of the recent molecular methods namely the Xpert MTB/RIF is a fully automated real time PCR
system that detects *M. tuberculosis* (Mt) complex and rifampicin resistance directly from clinical samples. This assay has been extensively evaluated in the diagnosis of pulmonary tuberculosis. The purpose of this study was to test the efficiency of this assay for the rapid and reliable diagnosis of extra pulmonary tuberculosis (EPTB).

**Materials and methods:** This was a pilot prospective blinded study done on 60 samples received in the department of Microbiology from patients with signs and symptoms of EPTB. All the samples were tested by Xpert MTB/RIF as well as routine conventional smear and culture.

**Results:** Overall the sensitivity, specificity, PPV and NPV of Xpert MTB/Rif assay in detecting *M. tuberculosis* from clinical samples was 86%, 100%, 100% and 83% respectively. In the drug susceptibility testing (DST) there was 100% correlation between rifampicin susceptibility pattern between Xpert MTB/RIF assay and DST by MGIT/LJ method. The mean turnaround time by Xpert MTB/ Rif assay was 2 hours when compared to culture and DST by LJ and MGIT systems were 46 days and 15 days respectively.

**Conclusion:** The Xpert MTB/RIF is a rapid and reliable diagnostic assay for detection of *Mycobacterium tuberculosis* and rifampicin resistance in EPTB samples.

**P33**

**Occupational transmission and prevention of HBV among health care workers in a tertiary care center**

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*BMC Infectious Diseases* 2012, 12(Suppl 1):P33

**Background:** Every day health care workers are exposed to dangerous and deadly blood borne pathogen like HBV. HBV is 100 times more likely to be transmitted after a percutaneous exposure through infected blood than HIV. Hepatitis B infection can be prevented by vaccination.

**Aim of the study:** To study the effect of vaccination program among the health care workers of Sri Ramachandra University (SRU), Porur, Chennai. It is mandatory for all health care workers of SRU to be vaccinated against HBV at the time of appointment, producing anti-HBs titer is mandatory. They are advised for re-vaccination according to their anti-HBs titer.

**Methodology:** This study was conducted among the health care workers of SRU who had needle stick injury for a period of 1 year (1st November 2010 to 31st October 2011). The blood samples were processed for serology screening by Micro Particle Enzyme Immuno assay (MEIA) followed by ELISA. The results were studied.

**Result:** 85 samples from health care workers who had needle stick injury were processed. The samples were from doctors (38%), nurses (20%), technicians (7%), housekeeping staff (23%), and students (10%). All the samples of injured persons were found seronegative by both methods, anti-HBs titer was found to be protective.

**Conclusion:** Strict HBV vaccination program and monitoring the anti-HBs titer were effective in prevention of HBV transmission by needle stick injury to health care workers of SRU. This can be implemented in all health care organizations for the benefit of health care providers.

**P34**

**Study of changes in lipid profile and fasting blood glucose in protease inhibitor exposed HIV/AIDS patients in School of Tropical Medicine, Kolkata**

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*BMC Infectious Diseases* 2012, 12(Suppl 1):P34

**Background:** The national second-line Anti retroviral Therapy (ART) programme was started in Kolkata in December 2008. It included a combination of Tenofovir, Lamivudine and Ritonavir boosted Lopinavir ± Zidovudine. Dyslipidaemia and increased fasting blood sugar (FBS) often complicate protease inhibitor-containing ART. Thus a prospective study was designed to observe the above changes.

**Methods:** The data of 48 patients, on protease inhibitor for one year were analyzed. Body Mass Index (BMI), grip strength (GS), Triceps skin fold (TSF), 24 hour dietary recall, serum triglyceride (TG), total cholesterol (TC), HDL, LDL, VLDL and FBS were estimated for all patients at baseline, 6 months and after one year.

**Results:** There was a significant increase in TG, TC and VLDL levels at 1 year as compared to baseline (p=0.013, 0.00 and 0.00 respectively) whereas LDL significantly increased at 6 months only (p=0.029). HDL decreased significantly at 6 months (p=0.019). TSF significantly decreased both at 6 and 12 months (p=0.00 and 0.00 respectively). The BMI and GS showed a significant increase at both 6 months (p=0.001, 0.000 respectively) and 1 year (p=0.005 and 0.00 respectively). Four patients with normal baseline FBS and one with impaired fasting glucose progressed to overt diabetes (FBS > 124 mg/dl) at 12 months. No significant change was noted in energy and protein intake of patients.

**Conclusion:** There is an increased incidence of dyslipidaemia and unmasking of diabetes related to protease inhibitor in this cohort. There has been an improvement in nutritional status as shown by BMI and GS.

**P35**

**A comparative study of Pap smear findings among HIV positive and negative women at Government Hospital of Thoracic Medicine (GHTM), Tambaram**

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*BMC Infectious Diseases* 2012, 12(Suppl 1):P35

**Background:** Cancer cervix is a major health problem in India, accounting for 26.1-43.8% of all cancers in Indian women. Dysplasia on Pap smear has been reported in 15-40% of HIV positive women which is 10-11 times higher than those observed among HIV negative women. Patients with persistent inflammatory Pap smears harbor high proportion of HPV infection and cervical intraepithelial neoplasia (20.9%). The purpose of the present study was to compare Pap smear findings in HIV-infected and uninfected women at GHTM and to correlate the Pap smear abnormalities among HIV positive women with their immune status.

**Methodology:** All women (18-45 years of age), admitted at GHTM, between July-Sept. 2010, consented were selected and Pap smear was performed. Smears were reported by a pathologist blinded to the HIV status of patients. Results were analyzed by using PASW 18 version.

**Results:** Total 300 samples were collected in which 204 were HIV positive and 96 were HIV negative. HIV positive women (58.8%) had more Pap smear abnormalities such as cervical dysplasia (3.92%), inflammatory smear (51.96%), infection (2.94%) (p=0.010) when compared with HIV negative women (42.75%). Among HIV positives 63.93% had CD4 count less than 250 (p=0.048), and 64.3% had coexisting opportunistic infection (p=0.04). On speculum examination, cervicitis was present in 63.3% of HIV positive women with abnormal pap smears (p=0.004).

**Conclusion:** This study showed a high percentage of inflammatory Pap smears (51.96%) among HIV positives. Hence these patients will require a follow up Pap smear and colposcopy and biopsy if inflammation is persistent to exclude cervical cancer.

**P36**

**Tuberculous Hansen’s – a case report**

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*BMC Infectious Diseases* 2012, 12(Suppl 1):P36

**Background:** Leprosy also known as Hansen’s disease is still prevalent. This case is presented to highlight the fact that it can occur even in immunocompetent individuals in the form of Tuberculous Hansen’s and for...
the fact that a single bacillus was identified on tissue sections stained by the Fite-ferako technique, which is rare in tuberculoid Hansen's. Case report: A fifty-three year old male patient presented with a hypopigmented patch over the left forearm measuring four centimeters for the past one month. He had numbness also over the patch. A skin biopsy was taken from the patch and sent for histopathological examination.

Background: Early institution of therapy has been advocated worldwide. However, until recently, NACO guidelines recommended therapy in HIV positive patients with CD4 count <250/µL. In this study we observed the clinical and immunological disease progression for a year in treatment naive patients with CD4 count between 250-500/µL.

Method: 150 treatment naive adults PLHIV with CD4 count between 250-500/µL in WHO clinical stage 1 or 2 were included in the study and enrolled into 2 groups at 2:1 ratio and followed for 1 year. Group A with CD4 count between 250-350/µL & Group B with CD4 count between 350-500/µL. At the end of study each group was evaluated for number of opportunistic infection, ART conversion, conversion to stage III and IV, lost to follow up (LFU) and death. Statistical analysis was done by SPSS 16.0 version. Data were expressed as mean ±SD for continuous variable and percentage for categorical variable. An independent sample t-test was used to detect differences in clinical and laboratory results.

Result: As compared to Group B, Group A had significantly higher number of opportunistic infection (27% vs 8%, p=0.006), ART conversion (30% vs 10%, p=0.012), death (9% vs 0%, p=0.021) and LFU (20% vs 6% p=0.037).

Conclusion: This study reinforces the need of early initiation of treatment in patients with CD4 <350/µL.

Background: Oral lesions among HIV seropositive individuals in an era of generic HAART: markers of HAART efficacy? Janani Vasudevan1, Vaishnavi Sivasankar1, Umadevi K Rao1, Nagalingeswaran Kumarasamy1, Kannan Ranganathan1

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BMC Infectious Diseases 2012, 12(Suppl 1):P39

Background: Oral lesions among HIV seropositive individuals have been established as a clinical feature of human immunodeficiency virus (HIV) seropositivity. Treatment with Highly Active Antiretroviral Therapy (HAART) has changed the course of infections with HIV. This study correlates the oral lesion prevalence in HAART and non-HAART groups in a cohort of HIV seropositive patients in Southern India.

Methods: The study group consisted of 3485 HIV seropositive patients who were not on HAART who reported to the dental clinic of YRG CARE, Chennai, India. Oral lesions were diagnosed clinically based on EC Clearinghouse criteria and the data was analyzed using SPSS software.

Results: In the study population, 92.3% of individuals had acquired the infection through heterosexual contact. The mean age of infected males and females was 35±8 and 30±8 years respectively and the mean CD4 count in males and females was 385.5±226.76 and 284.3±278.28 cells/µL respectively (p<0.05). Patients having CD4 count <200 (n=908) had more number of oral lesions than patients with CD4 count >200 (p<0.01). Patients on HAART (n=1042) had a lesser number of lesion than patients not on HAART (p<0.01). Of the lesions, candidiasis was predominantly seen in the non-HAART group (19.7%) as compared to the HAART group (11.7%). Patients who were not on HAART showed a higher prevalence of pseudomembranous candidiasis (12.8% vs.7.9%, p<0.01) and angular cheilitis (6.0% vs.3.7%, p<0.01).

Conclusion: Patients on Highly Active Antiretroviral Therapy (HAART) demonstrate fewer oral lesions, which can serve as markers of treatment efficacy. Long term follow-up studies are necessary to validate these results.
Methods: Study was conducted in ART centre, IMS, BHU. Patients who failed on first line ART (NACO criteria) were included in the study after consent. Virological, immunological and clinical response was assessed at 24 weeks. Independent t-test (Mann-Whitney U test was used for making comparison among the independent groups. Chi-square test was used to test the association between categorical variables. Spearman correlation coefficient was used to measure the degree of association between two variables.

Results: Out of 78 patients, only 39 (50%) had virological suppression (viral load<47 copies/ml) and 20 (25.6%) had partial response (viral load >47-1000). 10 (12.8%) had viral load >1000 copies/ml and 8 (10.3%) patients died before 6 months and 1 patient was lost to follow up. Median increase in CD4 count was 133 cells /ml (IQR : 46-498). Among patients who expired 90% had clinical failure at baseline, 50% and 18.75% were in stage IV and III respectively.

Conclusion: Currently recommended second line regimen appears to be inadequate as large proportion of patients failed to achieve the desired virological suppression. Our finding implies a relook at the present second line regimens recommended by NACO.

P41

Treatment seeking behavior of people with malaria and households’ expenditure incurred to it in a block in endemic area in Assam, North East India
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BMC Infectious Diseases 2012, 12(Suppl 1):P41

Background: The 60-90% preponderance of Plasmodium falciparum malaria places an economic burden in the community. The objective of the study was to know the treatment seeking behavior of people with malaria and households’ expenditure incurred to it in a block in endemic area.

Methods: In a cross sectional survey, all 210 diagnosed malaria patients within one month were interviewed through a pre-structured interview schedule during the high transmission season in pre, post and monsoon period in 2010.

Results: During the last episodes of malaria, 58.5% sought treatment from government health facilities out of which 41.4% went to allopath, 17.1% went to community health workers, 25.3% went to private practitioners of which 12.9% went to tea garden doctors, 8% to other facilities, 7.6% to traditional healers, 9% to homeopath and 4% to none. Self treatments were taken by 59% patients. Plasmodium falciparum affected 55.2% patients, Vivax 41.4% and mixed infection 3.3%. The median expenditure incurred on treatment was Rs21, on preventive action were Rs150 and daily wage loss was Rs100. The SC, ST (65.7%), farmers and daily wage laborers were going less to government facilities. The household expenditure was mainly associated with self treatment and repeat malaria.

Conclusion: The people of lower socio-economic group utilized more government health facilities for malaria treatment. Self treatment and repeated malaria had an impact on household expenditure. Improvement in quality health care delivery in public sector and IEC activities in the community would empower for maximum utilization of government health facilities.

P42

Seroprevalence of hepatitis C virus markers in multi-transfused children with beta-thalassemia
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BMC Infectious Diseases 2012, 12(Suppl 1):P42

Background: To study the seroprevalence of hepatitis C virus in multi-transfused children with β-thalassemia and compared with non transfused children and healthy controls. β-thalassemic children fail to thrive, with growth and development retardation and suffer microcytic hypochromic anemia. Since regular blood transfusions are given to maintain haemoglobin at a safe level, these children are at a high risk of acquiring hepatitis C virus through transfusions.

Methods: Study group, consisted of children 2-13 years with β-thalassemia and received more than 5 transfusions. Matched control group consisted of 30 children with β-thalassemia and no transfusion. Control group, consisted of 30 normal healthy children serum samples from all three groups were tested for antibodies to hepatitis C virus using commercial ELISA kits.

Results: Study showed 32% anti H hepatitis C virus positivity in multi-transfused and 0% in matched and healthy control groups. Hepatitis C virus infection showed a significant increase in relation to the number of transfusions received.

Conclusion: This observation is of great concern, as these children are at a risk of developing chronic hepatitis, cirrhosis and hepatocellular carcinoma. Since vaccination against hepatitis C virus is not available, highly sensitive and specific screening methods of donor blood in blood banks must be made mandatory.

P43

Novel acyclic nucleoside analogues as inhibitors of HIV-1 RT
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Background: Nucleoside reverse transcriptase inhibitors (NRTIs) were the first drugs introduced for treatment of human immunodeficiency virus-1 (HIV-1) infection. These NRTIs may be cyclic or acyclic analogs of natural nucleosides. Both these analogs interact at active site on HIV-RT and compete with indigenous nucleosides/nucleotides, and thus, divert enzyme activity in manmade direction. All NRTIs follow three phosphorylation steps that convert the parent compound successively to triphosphate. These 5’ triphosphates act as alternate substrate for HIV-RT, and lead to chain termination when incorporated into the DNA chain as they don’t provide the 3’-OH function.

Method: Designing of acyclic allylic nucleoside analogs, which act as NRTIs against HIV, involves both the computational and synthetic methods. Designing is done keeping the Lipinski’s Rule of Five in focus and SAR studies were performed using DS 3.0 software. The ADMET descriptor and TOPKAT protocol available in DS 3.0 were used to predict these properties. The Lipinski’s Rule of Five was also used to determine the biological activity or druglikeness of the designed inhibitors.

Result: All acyclicuracil analogues formed 3-10 bonds with amino acids constituting the dNTP site on HIV-RT. The amino acids that interact with these molecules are Glu44, Lys46, Lys65, Arg72, Asp110, Asp113, Glu151, Asp185, Pro217, His221, Lys223 through H-bonding and n-n interaction.

Conclusion: On the basis of SAR studies, acyclic allylic analogs of uracil bearing carbonyl and sulphonyl groups at N-3 position are expected to be probable lead molecules against HIV-RT. Biological screening is under process.

P44

Response of Caenorhabditis elegans during subsequent infections with Gram positive and Gram negative bacteria
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Background: The nematode Caenorhabditis elegans is one of the popular model hosts for the study of the evolutionarily conserved mechanism of microbial pathogenesis and innate immunity. C. elegans can be effectively used to study the dynamics of polymicrobial infections. Proteus mirabilis, an opportunistic pathogen, does not cause death in C. elegans. In this study the C. elegans was pre-infected with Staphylococcus aureus to make the C. elegans immunocompromised to study the effect of P. mirabilis in the host.

Methods: This study involved in investigation of impact of subsequent infections at both physiological and molecular levels using C. elegans by killing assays and real time PCR analysis.
Results: The study revealed that 12 h of S. aureus and 80 h of P. mirabilis subsequent infections reduced the life-span of 80% of the infected nematodes. Real time PCR analyses indicated the regulation of innate immune regulatory genes, lysosome, CUB-like proteins, neutrophide-like factors, transcription factors of MAP kinase and daf-2, daf-16, insulin-like signaling pathways and C-type lectin family members during polymicrobial infections, indicating possible role and contribution of the above players during host immune response against subsequent infections.

Conclusions: Our findings demonstrate that the vulnerability of a host is an integral part of the S. aureus infection that enables the bacteria to subvert the host immune system, which can lead to the P. mirabilis to exert its pathogenicity in the host C. elegans.

P45
Detection of fusidic acid resistance determinants among Staphylococcus aureus isolates causing skin and soft tissue infections from a tertiary care centre in Chennai, South India
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Background: Fusidic acid (FA)- an inhibitor of protein synthesis has been used for treating superficial and some systemic infections caused by Staphylococcus aureus. Fusidic acid resistance in S. aureus has been reported throughout the world with prevalence ranging from 0.5% to 50% and is due to i) point mutation in bacterial fusB or fusE gene and ii) by acquired FA resistance determinants fusD, C and D. Indian report of fusidic acid resistant S. aureus (FRSA) is based on phenotypic detection. Hence, this study was done to detect acquired FA resistance determinants.

Methods: The study included 54 isolates of S. aureus collected from skin infections between Jan to Mar 2011 from a tertiary hospital in Chennai. MRSA was screened by cefoxitin disc diffusion method and PVL-MRSA detection was done by multiplex-PCR. FA resistance was screened by disc diffusion method and acquired resistance determinants were detected by multiplex-PCR.

Results: Of the 54 S. aureus isolates, 32(59.2%) were found to be MRSA. A total of 13(24.1%) isolates were found to carry pvl gene of which 4 were MRSA. Two of the 54(3.7%) isolates were found to be FRSA and harbored fusC gene. Both FRSA isolates were from non-hospitalized patients and they were using FA for topical treatment.

Conclusion: We report for the first time in India the presence of acquired FA resistant determinant fusC gene in community isolate of methicillin susceptible S. aureus. Indiscriminate use of FA needs to be avoided to prevent the emergence of FRSA.

P46
Evaluation of HIV-1 polymerase chain reaction (PCR) technique using dried blood spots (DBS) for diagnosis of perinatal transmission of HIV
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BMC Infectious Diseases 2012, 12(Suppl 1):P46

Background: Effective health care delivery to the majority of perinatally exposed infants is hampered by lack of access to accurate HIV diagnosis in infancy. Polymerase chain reaction is the most sensitive test to diagnose HIV-1 infection in children born to HIV seropositive mothers. The purpose of this study was to assess the feasibility and accuracy of using dried blood spot (DBS) technology in performing HIV-1 DNA PCR using Roche Amplicor HIV-1 DNA PCR version 1.5 for diagnosis in children less than 18 months of age.

Materials and methods: This was a prospective observational study. 41 newborn infants of HIV-infected mothers were recruited and in addition 24 HIV-infected mothers, served as positive controls. DNA was extracted from filter paper using chlex resin and amplified using Roche Amplicor HIV-1 DNA PCR test. Sensitivity and specificity of the DBS PCR analysis was analyzed by comparing it with the results of PBMC’s (peripheral blood mononuclear cells).

Results: Out of 66 DBS samples tested, 31 were positive, 35 negative and there was no indeterminate result. Whereas using PBMC, 32 samples were positive, 34 negative and 4 samples which were indeterminate initially were negative on repeat testing. All mothers were positive by both from DBS and PBMC’s. Overall sensitivity and specificity of DBS using Roche Amplicor DNA PCR was 97% and 100% respectively.

Conclusion: PCR performed using DNA extracted from filter paper using chlex method is simple, sensitive and specific and can be used in resource limited settings.

P47
Therapeutic implications of nanoencapsulated M. avium / HIV drugs against experimental tuberculosis in mice
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Background: Therapeutic management of Mycobacterium avium infection is inadequate due to patient non-compliance, lengthy treatment regimen, multidrug associated toxic side-effects etc. particularly in AIDS patients who are at a greater risk of developing mycobacterial infection. This study was designed to evaluate chemotherapeutic potential of poly D, L-lactide-co-glycolide nanoparticles against M. avium infection in mice.

Methods: Drug loaded nanoparticles were prepared by double emulsification and characterized for their size, surface morphology and sustained drug release. Pharmacokinetics of free and nanoencapsulated drugs were evaluated after single oral dose administration and therapeutic efficacy was assessed in M. avium infected mice after 4 weeks of chemotherapy.

Results: Sustained release of various drugs was observed for 5-7 days as compared to 24h for free drugs in plasma and various tissues. Eight weeks of chemotherapy resulted in significant clearance of bacilli from lungs and spleen of M. avium infected mice as compared to untreated controls. 8 doses of PLGA nanoencapsulated M. avium drugs depicted an equivalent therapeutic effect as that of 56 doses of daily administered oral free drugs which was evident from cfu enumeration data and lung histopathology. Furthermore, nanoencapsulation was observed to lessen the adverse drug interactions between anti-retroviral and anti-M. avium drugs.

Conclusion: PLGA nanoparticle based drug delivery system showed great potential to produce sustained release of anti-HIV / M. avium drugs. These studies hold promise to reduce the frequency of drug dosages as well as alleviate adverse drug interactions during the course of M. avium and HIV therapy.

P48
A microbiological study of neonatal conjunctivitis in two hospitals in Tehran, Iran
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Background: Conjunctivitis during the neonatal period is accompanied by diffuse conjunctival injection; it is usually acquired and may result in serious eye damage. This study is to define the prevalence of neonatal conjunctivitis and to identify the causative agents of ophthalmia neonatorum in two university hospitals from 2008-2009.

Methods and materials: All neonates admitted in the neonatal department during the study period were examined for the presence of conjunctivitis. Two swab specimens containing epithelial cells of the conjunctiva were collected from newborns presenting with conjunctival inflammation. Laboratory diagnosis was based on bacterial culture and
Gram staining. The isolated bacteria were identified using standard procedures. For identifying Chlamydia trachomatis we used PCR and cell culture.

Results: Of the 2253 neonates, (age range 1-30 days), clinical findings of conjunctivitis were found in 241 cases, (10.7%). The most commonly isolated bacteria were Coagulase Negative Staphylococci, (N=130, 53.9%); Chlamydia trachomatis was the second most common cause of acute neonatal conjunctivitis, (n=40, 16.6%). Bacterial cultures were negative in 47 neonates (19.5%) despite clinical signs of conjunctivitis. The median age at presentation for bacterial culture positive was day 8 of life.

Conclusion: Neonatal conjunctivitis is prevalent in newborns; Gram Positive Cocci and Chlamydia trachomatis are the most common causative organisms.

P49
Gag-Vpu cross talk modulating HIV-1 envelope incorporation and infectivity in cell-type dependent manner
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BMC Infectious Diseases 2012, 12(Suppl 1):P49

Introduction: HIV-1 Vpu plays important role in enhancing virus release and CD4 down-modulation. We investigated the effect of Vpu-Gag cross talk on envelope incorporation and assembly in different cell types including primary cells.

Methods: We introduced Vpu start codon mutation and point substitution in p17 Gag matrix (L30E) mutation in HIV-1 pNL4-3D through PCR. Pregeny viruses were produced in two cell-types: 293T and HeLa by transfection. Infectivity potential of Vpu+/Vpu- viruses carrying Gag (L30E) mutation was assessed in TZM-bl cell line and their replication potential in peripheral blood mononuclear cells (PBMC) and monocyte-derived macrophages. The effect of mutation on virus release, envelope incorporation and infectivity was determined by RT ELISA and Western blot.

Results: The amount of virus release from 293T and HeLa cells was similar in Vpu+/Vpu- constructs carrying Gag (L30E) mutation but the infectivity potential of viruses varied, showing enhanced infectivity of Vpu-.) Gag L30E viruses produced from 293T cell and HeLa cells. Envelope incorporation assay using 293T cells revealed that Vpu+ viruses with L30E mutation showed inefficient incorporation of HIV-1 envelope on cell-free virions whereas viruses with Vpu+/ Vpu- L30E mutation resulted in efficient envelope incorporation and thereby increasing their infectivity potential. While similar results were obtained with PBMC and macrophages as found with 293T and HeLa cells, these effects were found to vary in different cell types.

Conclusion: HIV-1 envelope incorporation and infectivity is dependent on cross talk between p17 Gag, Vpu andEnvelope and the effect of Gag p17 mutation on envelope is Vpu-mediated.

P50
SDF-1 gene polymorphism and CCL3L1 gene copy number and susceptibility to HIV-1 / AIDS among Indians
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BMC Infectious Diseases 2012, 12(Suppl 1):P50

Background: Stromal derived factor (SDF-1) is a natural ligand for CXCR4 and chemokine (C-C) motif ligand 3-like 1 (CCL3L1) is for CCR5 HIV coreceptors. The individual role of the SNP in 3′ untranslated region of SDF-1 (SDF1-3′) and low copy number of the CCL3L1 gene in determining susceptibility to HIV infection is well documented. The aim of the present study was to analyze the synergistic effect of the SNP in SDF-1 gene and CNV in CCL3L7 gene influencing the susceptibility to HIV-1/AIDS in Indians.

Methods: This study involved the assessment of 105 healthy control individuals and 44 HIV-1 patients for the SDF-1 gene polymorphism by PCR-restriction fragment length polymorphism (RFLP) and CCL3L1 gene copy number (CN) by real-time PCR.

Results: In order to assess the synergistic effect of the SDF1-3′A polymorphism and CCL3L1 CN, SDF-1-3′A allele and CCL3L1 > 2 copies conferring a protection to HIV-1 were considered as reference combination. The odds ratio (OR) was 0.97 (95% CI 0.30 - 3.13; p = 0.597) for SDF1-3′A, CCL3L1 > 2 copies and 0.73 (95% CI 0.23 to 2.33; p = 0.408) for SDF1, CCL3L1 > 2 copies and 0.93 (95% CI 0.22 to 2.20; p = 0.555) for SDF-1′A, CCL3L1 ≤ 2 copies combinations as compared to SDF-1′A, CCL3L1 > 2 copies.

Conclusion: Our analyses suggest that a combination of SDF-1′A and lower copy number of CCL3L1 do not provide any discernible synergistic protection from HIV-1 infection.

P51
Highly conjugated curcumin analog based copper complexes towards tuberculosis: synthesis, characterization and antimycobacterial activity
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BMC Infectious Diseases 2012, 12(Suppl 1):P51

Background: Current first-line drugs for the treatment of tuberculosis consist of, i.e., isoniazid (INH), rifampin (RIF), ethambutol (EMB), pyrazinamide (PZA), and streptomycin (STR). Resistance to the first-line drugs causes treatment failure and necessitates the use of the drugs with a prolonged period of therapy. New anti-tuberculous agents, especially the ones with novel mechanisms of action are urgently required. Curcumin is a naturally occurring yellow pigment obtainable from the rhizomes of perennial herb Curcuma longa Linn., has been shown to act upon several important molecular targets in malignancy and inflammatory cascades and hence is used to treat various disorders including arthritis, Crohn’s disease, cardiovascular disorders, psoriasis, cancers, and other pathologies. However, poor water solubility and unsatisfactory pharmacokinetics of curcumin necessitate search for new curcumin analogs. In the present work, we described the synthesis and structural characterization of highly conjugated curcumin analogs (acetoacetanilide) Knoevenagel condensates, their Schiff bases, and corresponding copper conjugates.

Method: The anti- M. tuberculosis activities of the compounds were determined using the MABA assay method.

Results: The minimum inhibitory concentration of copper complexes has been performed against Mycobacterium tuberculosis strain H37Rv. It is observed that the MIC values of copper complexes (2-6 µg/ml) are slightly greater than the drug, ethambutol (1 µg/ml).

Conclusion: Copper complexes have higher anti-mycobacterial activity than ligands due to the presence of highly conjugated curcumin analog system containing two azomethine groups and redox properties of metal. Based on our studies, we conclude that copper complex may be a promising candidate against tuberculosis.

P52
Effects of HIV related stigma on the lives of persons living with HIV
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BMC Infectious Diseases 2012, 12(Suppl 1):P52

Background: Persons living with HIV are stigmatized throughout the world in varying degrees. PLHIVs experience stigma in two forms – internal and external stigma. Due to internal stigma, PLHIVs isolate themselves from the community and they do not even access essential health care services. Due to external stigma, PLHIVs are rejected by their loved ones and their community, unfairly treated in the workplace and are denied access to health services. The present study made an attempt to bring out the effects of HIV related stigma on the lives of PLHIVs in the Nilgiris.

Methods: To measure the existence of internal and external stigma 180 PLHIVs were selected. Various qualitative research methods such as focus group discussions and case study were adopted to gather necessary data.
Focus group discussions were conducted with 47 PLHIVs (11 male and 36 female). Case study highlights the in depth situation of the PLHIVs, hence eight PLHIVs were interviewed and gathered needed information with their permission.

Results: Ninety four percent of respondents mentioned about the feelings of rejection. Ninety two per cent PLHIVs conversed about the guilty feeling of being a PLHIV. Ninety four percent of selected PLHIVs mentioned that they were emotionally affected due to the discriminatory activities of their own family members. Children of PLHIV parents experienced discriminatory practices in the school regardless of their HIV status.

Conclusion: The qualitative data obtained in this study substantiate the stigmaizing and discriminatory experiences such as denial of house for rent, denial of property and marital conflicts etc.

PS3

Socio-demographic profile of HIV/AIDS patients at ART centres in Chennai

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Background: To study the socio-demographic status of HIV patients in ART centres, Chennai, Tamilnadu.

Methods: After obtaining informed consent, a semi-structured questionnaire was administered to adult HIV patients attending ART centers, Chennai. Their demographic details, personal history, behavioral pattern were studied between June-October 2011.

Results: Out of 296 patients, 117 were male, 174 were female and 5 were transgender. 77.4% of them were between the age group of 18-41. Among all, 20.6% were illiterate and 79.4% were literate. Among the literates, 5.7% were graduates. In men, 77.8% were married. Among married males 5.1% were separated and 7.7% were widowers. In women, 97.7% were married. Among them 29.9% were widow and 9.8% were separated. 84.4% of male and 28.1% of female were employed. 53.9% of patients were graduates. In men, 77.8% were married. Among married males 5.1% were separated and 7.7% were widowers. In women, 97.7% were married. Among them 29.9% were widow and 9.8% were separated. 84.4% of male and 28.1% of female were employed.

Conclusion: Low socio-economic status with high risk behaviour and lack of awareness were prevailing among the HIV patients. Epidemiological studies should be carried out in various settings to understand the role of socio economic status to control the transmission of HIV/AIDS.

PS4

Factors affecting default among pre-ART patients in Eastern Uttar Pradesh

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Background: Retention of patients in HIV care is a challenge as they need continuous monitoring to prevent development of advanced disease. Unfortunately even after 8 years of rolling out of ART program, there is no data on pre-ART follow up. Hence this study was conducted to identify the factors leading to default during the pre-ART period.

Methods: This cross sectional study was conducted at ART centre, COE, BHU. All patients 18 years of age and above defaulted from pre-ART HIV care were included in the study. Defaulters were defined as any patients who missed their last appointment of CD4 count by more than one month (missed) & more than three months (lost to follow up). All these patients were traced telephonically and interviewed after taking consent. Statistical analysis was done by using SPSS version 15.0.

Results: Out of the 1532 patients registered in pre-ART care 367 were defaulters and 144 could be traced. Only 83 patients gave their consent for the interview, 73 were LFU and 10 were in the missed category. Default was common among females & patients with rural background. The main reasons for defaulting from pre-ART care were feeling of wellness 65.1%, and distance of health facility 61%.

Conclusion and recommendations: The study recommends that there should be regular updating of contact information. Counselling at ART centre should focus on importance of CD4 testing & its frequency. There is a strong need to start tracking of pre-ART patients enrolled in HIV care.

PS5

Assessment of hematological adverse drug reactions to antiretroviral therapy in HIV positive patients at Kasturba Hospital Manipal

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BMC Infectious Diseases 2012, 12(Suppl 1):P55

Background: HIV-infected patients have a higher risk of developing hematological adverse drug reactions (ADRs) than the general population and have a significant impact on patients’ current and future care options. The study was to determine the causality, the incidence rate, severity pattern of occurrence of hematological ADRs associated with highly active antiretroviral therapy in HIV positive patients.

Methods: Prospective observational study conducted at medicine department, Kasturba Hospital over a period of 6 months. Enrolled HIV positive patients were intensively monitored for hematological ADRs associated with fixed dose of highly active antiretroviral therapy. Causality of adverse drug reactions was assessed by using WHO probability scale and also with Naranjo’s algorithm.

Results: Monitoring of 70 HIV positive patients (58 males and 12 females) with fixed dose drug combination of antiretroviral therapy by active pharmacovigilance identified 47.3% cases of anemia, 15.7% cases of leucopenia, 21% cases of pancytopenia, 5.2% of eosinophilia, 10.5% cases of bicytopenia. The incidence rate of hematological adverse drug reactions was 37.9% and most of the reported ADR’s were definitely predictable and preventable. Fifty percent of these ADR’s were lead to hospitalization and seventy percent of these ADR’s were of moderate in severity. Hematological ADRs were highest with Zidovudine + Lamivudine + Nevirapine (68.4%) and Zidovudine + Lamivudine + Efavirenz (31.29%) combinations.

Conclusion: With the increasing access to use of highly active antiretroviral therapy in India, clinician must focus for routine monitoring of hematological investigations for early detection and prevention of adverse effects associated with highly active antiretroviral therapy.

PS6

A confocal microscopic study on biofilm formed by Pseudomonas spp. isolated from lower respiratory tract infection from HIV and non-HIV populations

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Background: Pseudomonas is one of the predominant microorganisms of chronic lung infections. Bacteria growing in biofilm often develop
multicellular, three-dimensional structures known as biofilm. *Pseudomonas* colonizes the lungs by forming biofilm micro colonies throughout the lung. In this study, using *Pseudomonas* spp. artificial biofilm was grown on flow cell chamber and images were taken using Confocal Laser Scanning Microscope (CLSM), to establish an experimental model for artificial biofilm in vitro.

**Methods:** Out of 71 isolates analysed, 45 were HIV and 24 non-HIV isolates. 19 (26.76%) produced biofilm. 6/45 (13.33%) HIV isolates and 13/24 (54.17%) non-HIV isolates were biofilm producers. 4 strains were taken to study the in vitro biofilm formation using flow cell chamber and their 3D structure and architectures were studied using CLSM. Biofilm formation was monitored at different time intervals (3, 72 and 144 h). For each time interval, one channel was stained with acridine orange dye and the images obtained were quantitatively analyzed by COMSTAT.

**Results:** A time lapse study of various time intervals (3, 72 and 144 hrs) were taken to study the biofilm formed by the *Pseudomonas aeruginosa*. We therefore used a computer program COMSTAT for quick and easy analysis of the biofilm image data which calculates a number of variables characterizing the three-dimensional structures.

**Conclusion:** Biofilm have been established as a main cause of infections due to the increased chemo resistance compared with bacteria in suspensions. Hence it is necessary to characterize the developmental steps leading to the formation of the *P. aeruginosa* biofilm.

**P57**

**Resistance pattern of Acinetobacter spp. isolated from various clinical samples in and around Kanchipuram**

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**BMC Infectious Diseases** 2012, 12(Suppl 1):P57

**Background:** For the past two decades *Acinetobacter* spp. has been emerged as an important pathogen in various infections. Emergence of multi drug resistance limits the therapeutic option. So this study is aimed to determine the antibiotic resistance pattern of *Acinetobacter* spp. from various clinical samples.

**Methods:** The study included a total of 1516 samples of which 892 were from pus and 624 were from sputum collected from patients at Meenakshi Medical College and Research Institute, Kanchipuram from Nov 2010 to August 2011. Samples were processed and identified according to standard protocol. The *Acinetobacter* spp. isolates were tested for antibiotic resistance against 16 antibiotics by Kirby Bauer disc diffusion method (CLSI guidelines).

**Results:** Out of 1516 samples collected, 50 (3.3%) *Acinetobacter* spp. was isolated. Among which 35/50 (70%) were from pus, 15/50 (30%) were from sputum. All the isolates were found to be 100% resistant to ampicillin, 76% resistance to co-trimoxazole ceftaxime and ceftazidime, 64% to doxycycline. Ciprofloxacin and gentamicin showed 58% resistance, followed by netilmicin, amikacin and tetracycline with 57%, 50% and 46% resistance. Ofloxacin, sparfloxacin and levofloxacin showed 33%, 21% and 17% resistance. Least resistance was observed for amoxyclav, ceftriaxone/tazobactam and imipenem with 11%, 5% and 5% resistance respectively. Out of 50 isolates 31 (62%) were found to be multi drug resistant.

**Conclusion:** Multi drug resistance among *Acinetobacter* spp. is of great clinical importance which provacates the need to screen and formulate appropriate antibiotic policy for the hospitals and prevent further development and spread of resistant strains.

**P60**

**Silver nanoparticles as an antibacterial agent for endodontic infections**

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**BMC Infectious Diseases** 2012, 12(Suppl 1):P60

**Background:** Bacteria play an essential role in the initiation, progression, and persistence of dental infections. Therefore endodontic therapy aims to eliminate bacteria from the infected root canal and prevent reinfection. **Materials and method:** Silver nanoparticles have gained more attention owing to their broad spectrum of antibacterial activity and low cost of manufacturing. In the present study, starch coated silver nanoparticle were synthesized and characterized by SEM/EDX and UV/Vis spectroscopy.
Starch coated nanosilver was tested for its antibacterial activity against various microorganisms that are commonly found in endodontic failures such as Enterococcus faecalis, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Acinetobacter baumannii, Candida albicans, Klebsiella pneumonia. The antibacterial activities were assessed in vitro by 1) Agar diffusion test (ADT) 2) MIC by spectroscopic method 3) efficacy assessment using dentinal tubule model at depths of 200 μm and 400 μm in extracted single rooted teeth.

Results: The results indicated that the synthesized starch coated nanosilver showed good bactericidal effect against a wide range of organisms. The efficacy study using human tooth model shows that there was a significant reduction in the adherence of Enterococcus faecalis to nanoparticles-treated dentin.

Conclusion: These experimental results highlighted the potential advantage of silver suspension in root canal disinfection and thereby reduces bacterial invasion into dentin. Hence this eco friendly starch coated silver nanoparticle could be developed as a potent antibacterial agent against a wide range of microorganisms to control and prevent the spreading and persistence of endodontic infections.

P61 Viral hepatitis amongst Saharia: a primitive tribe of Madhya Pradesh
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Background: The prevalence of viral hepatitis in tribal areas of India mostly remains unknown. Saharia is one of the primitive tribes of Madhya Pradesh and live primarily in the Sheopur and Shivpuri districts. The purpose of the present study was to assess the prevalence of hepatitis viruses in Saharia tribal community of central Indian state of Madhya Pradesh.

Methods: A cross sectional study was carried out to determine the point prevalence of different hepatitis viruses amongst Saharia tribal community of Madhya Pradesh, central India. After obtaining the informed consent, blood samples (5 ml each) were collected from them. Serum was separated on site, aliquoted and transported to the laboratory maintaining the cold chain. The markers of various hepatitis viruses were detected using semi quantitative ELISA kits. The specimens found positive for HBsAg were tested for HBV DNA by Polymerase Chain Reaction (PCR).

Results: A total of 173 blood samples were collected. The prevalence of HBsAg and anti HBs was found to be 5% and 33% respectively. The prevalence of anti HCV was 1%. Anti HAV antibodies were present in 165 samples (99%). The prevalence of anti HEV was found to be 40%. 4 samples were found positive for HBV DNA by real time PCR.

Conclusion: The findings of the study indicate that viral hepatitis infection is an important problem in saharia tribal primitive community. Control measures including IEC strategies are necessary among them.

P62 Streptococcus invasive locus (sil) in Group A Streptococcus causing non-invasive infections in Chennai, South India
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BMC Infectious Diseases 2012, 12(Suppl 1):P62

Introduction: Group A Streptococcus (GAS) causes diseases ranging from superficial skin & throat infections to severe life-threatening diseases. Streptococcus invasive locus (sil) is a virulence gene responsible for pathogenesis of GAS. This study intends to detect the presence of sil gene among the non-invasive GAS and its association with toxin genes, erythromycin resistance genes, and emm types.

Methods: A total of 85 GAS isolates (43 pyoderma, 18 pharyngitis, 24 carrier) were screened for presence of sil C&D gene, toxin genes (speA, speB, spec, speC, smaZ, speH, speJ, ssa, speF), and erm genes by PCR. emm typing was done by emm gene amplification and sequencing.

Results: Among 85 isolates, 20/85 (23.5%) were positive for sil C and 27/85 (31.8%) isolates were positive for sil D. Both sil C&D were present in 20/85 (23.5%) isolates, whereas 58/85 (68.2%) isolates were negative for both sil C&D. Comparing the presence of sil C&D among the isolates from different sources, no significant difference (p=0.05) was found. There was no significant differences between the toxin gene profile and presence of emm genes between sil-positive/negative isolates (p=0.05). Thirty nine different emm types were observed among the 85 GAS, reflecting a diversity of 45.88%. emm types harbouring sil were emm9B.0b(5), emm82.1(3), emm74.0(2), emm80.0(1), emm95.0(1), emm105.I(1), emm11.0(1), emm44.0(1), emm55.0(1), emm66.0(1), rt2460.1(1), st36735.0(1), stG652.0(1). Many of these emm types were also found among the sil-negative strains.

Conclusion: 23.5% of the non-invasive GAS harboured sil. There was no specific association of sil genes with toxin genes emm genes, emm types or source of isolation.

P63 Bacterial vaginal infections in diabetic and non-diabetic women
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BMC Infectious Diseases 2012, 12(Suppl 1):P63

Background: Vaginal infection is a common disease of women. After 40 years, there is a fall in estrogen production. An estrogen deficient vagina as well as the immunocompromised status due to diabetes can lead to growth of abnormal flora which may in turn lead to infections. Bacterial vaginal infections are often least understood and empirical antifungal therapy for any vaginal infection without high vaginal swab culture is still in practice. The aim of the study is to analyze the prevalence of bacterial vaginal infections in diabetic and non-diabetic women.

Methods: Fifty diabetic and fifty non-diabetic women of age 40-70 years were randomly selected from the patients attending SBMCH, Chennai. High vaginal swab specimens were collected from them and cultured aerobically and anaerobically. Biochemical tests were performed and the microorganisms isolated. Antibiotic susceptibility pattern noted.

Results: The microorganisms isolated were bacteria, Candida spp., Trichomonas spp. The major pathogens were Escherichia coli (15%), Klebsiella pneumoniae (2%), Staphylococcus aureus (9%) and Candida (16%). Lactobacillii, Bacteroides fragilii and Peptostreptococcus spp. were the anaerobes isolated. E. coli, Laureus, Candida spp. were 18%, 12%, 18% reported in diabetic women and 12%, 6%, 14% reported in non-diabetic women respectively.

Conclusion: The prevalence of pathogenic bacteria and Candida is more in diabetic women than the non-diabetic women. Pathogenic bacteria are found as frequently as the Candida. Hence, the practice of empirical antifungal therapy without taking high vaginal swab needs to be revised. The use of appropriate antibiotics along with antifungal drugs may be beneficial.

P64 Characterization of Pseudomonas strains from sinusitis patients
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BMC Infectious Diseases 2012, 12(Suppl 1):P64

Background: Sinusitis affects significant percentage of population causing considerable long term morbidity. P.aeruginosa is responsible for about 13% of the sinusitis cases. Pseudomonas produces hemolysin, rhomnolipid, a membrane glycolipid, secretory pigments like pyocyanin and 1-hydroperanzine which can reduce ciliary beating and mucociliary clearance. Alginate functions as an adhesion, thereby preventing phagocytosis.

Methods: This study involved the characterization of 22 Pseudomonas strains isolated from endoscopic pus of 170 sinusitis patients. Tests for the production of haemolysin, protease, lipase, lecithinase, slime, leucocidin toxin were performed. Cell adherence property was also evaluated. Antibiotic susceptibility tests were performed.
Results: All the strains produced β-hemolysin. Coloured pigments ranging from bluish green to green, pink and purple were produced. Among the 22 strains, 18 were positive for proteases, 17 for lipases and 3 for lecithinase. All the 22 isolates produced leucocidin toxin, which killed all the leucocytes in 10 minutes. All the strains produced slime layers ranging from 20-35 mm height. The rate of cell adherence to the nasal epithelial cells depended on both the clinical strains and the individuals from whom nasal epithelial cells were taken. Clinical strains attached more efficiently (p=0.001) than the environmental strains. All the strains were sensitive to Amikacin, Gatifloxacin, Norfloxacin and Ofloxacin. To the antibiotics, Ceftriaxone, Gentamicin, Cefotaxime, Chloramphenicol and Pipercillin, 18%, 9%, 28%, 59% and 59% of the strains showed resistance respectively.

Conclusion: Thus it is evident that *P. aeruginosa* produces virulence factors, demonstrates resistance towards multiple antibiotics. Successful treatment necessitates a thorough knowledge of the prevailing bacteria.

**P65**

Comparative evaluation of screening and supplementary assays used in HCV diagnosis

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**Background:** Evaluation of serological assays for hepatitis C (HCV) antibody screening is crucial, as supplemental testing ([Nucleic Acid Testing & Recombinant Immunoblot Assay](http://www.biomedcentral.com/bmcinfectdis/supplements/12/S1)) to verify anti-HCV reactive results is not widely practiced, due to limited availability and high cost. The current study was undertaken from January to May 2011, to evaluate 4 serological assays for HCV diagnosis, viz-a-viz HCV RNA PCR and RIBA.

**Methods:** 87 patient specimens were screened for anti-HCV, using COBAS e411(eCLIA, Roche, Germany), Assy m (MEIA, Abbott, Germany), HCV Qualisa (ELISA, Qualipro, India) and HCV Tridot (Rapid Immunofiltration, J. Mitra, India), and for presence of HCV RNA using COBAS TQAMAN 48 analyzer (Roche). Specimens with discrepant results were referred internationally for RIBA.

**Results:** 58/87 (66.7%) specimens were anti-HCV reactive. 37/58 were HCV RNA PCR positive, indicating active HCV infection. Sensitivities of COBAS, Assy m, Qualisa and Tridot were 100%, 97.3%, 72.9% and 75.7% respectively. False negative anti-HCV results, obtained by Qualisa and Tridot, were seen in 10 and 9 patients respectively and could be attributed to the synthetic peptide coating in these kits. 6 of these patients were chronic renal failure cases, on hemodialysis. All kits showed specificity of 100%. False positivity was not observed, possibly because our study group comprised of patients with suspected HCV infection.

**Conclusion:** Anti-HCV assays on COBAS e411 (eCLIA) and Assy m (MEIA) platforms appear reliable. The study thus highlights the importance of using recombinant antigen based tests for anti-HCV screening. A strong need to conduct larger studies for performance evaluation of anti-HCV tests in specific patient subpopulations is felt.

**P66**

Oxidative stress index as a novel biochemical marker in tuberculosis; with therapeutic benefit of antioxidant supplementation

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**Background:** Severe oxidative stress has been reported in tuberculosis (TB) patients. There are no previous reports regarding oxidative stress index and supplementation of antioxidants in TB. The objective of this study was to assess the antioxidant status, lipid peroxidation and oxidative stress index in TB patients. Also to assess the antioxidant supplementation with antituberculous therapy in TB outcome.

**Methods:** The present cross-sectional study comprised of normal human volunteers (n=50), untreated TB patients (n=50), TB patients treated with anti-tuberculosis therapy (n=50) and TB patients treated with combination of antioxidant supplementation with ATT (n=50). Evaluation of lipid peroxidation was performed by estimating serum malondialdehyde, while the marker for total antioxidant capacity was performed by ferric reducing antioxidant power assay. The oxidative stress index was measured. The statistical analysis performed was One Way ANOVA.

**Results:** The oxidative stress index was increased significantly in untreated TB (P<0.05); these levels decreased significantly with clinical improvement in patients treated with combination of antioxidants and ATT when compared with patients treated with ATT only. The total antioxidant capacity was decreased significantly in untreated TB (P<0.05); these levels increased significantly in patients treated with combination of antioxidants and ATT when compared with patients treated with ATT only.

**Conclusion:** The oxidative stress index significantly increased in untreated TB patients and decreased in TB patients on ATT with antioxidant supplementation. Hence, oxidative stress index can be considered as a novel marker in TB patients. Also, the antioxidant supplementation in adjunct with ATT showed better improvement in outcome of TB.

**P67**

Emergence of HIV-1 drug resistance in antiretroviral therapy experienced perinatally infected children in South India

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**Background:** Over 25,000 HIV-infected children are currently alive and on highly active antiretroviral therapy (HAART) in India. Limited data are available on the efficacy of treatment and emergence of drug resistance (DR) in pediatric populations. In this study we aimed to characterize the pattern of drug resistance mutations (DRM) in a cohort of perinatally infected children.

**Method:** Blood samples were collected from 61 children who were on first line of therapy for ≥ 6 months. Patients’ demographic and clinical parameters were documented. Viral load was measured using Abbott m2000rt system, Germany. DR Genotyping (using an in-house method) was performed on those with viral load >1000 copies/ml.

**Results:** Among those who had been on ART for a median period of 24 months 51 children (83.6%) achieved virological suppression. There were 10 children with virological failure, and only two amongst these manifested immunological failure. Nine children (90%) had reverse transcriptase-related DRMs, and none had protease inhibitor-related DRMs. The most frequent NRTI mutation was M184V (n=9) followed by two thymidine analogues associated mutations (TAMs) M41L and T215Y (n=2). The most frequent nNRTI mutations were K103N (n=6) and Y181C (n=3).

**Conclusion:** Our study showed a high proportion of children achieving virological suppression with a mean duration of two years of ART. Children with virological failure and drug resistance and without immunological failure will likely promote DRM accumulation and may jeopardise second-line ART options. These data suggest that virological monitoring may help optimise regimen switch in children.

**P68**

Expression of ORF2 protein of TVT for development of EIA system

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**Background/aim:** Torque Teno Virus (TVT) is highly prevalent across the world. It is ssDNA virus having 4-5 ORFs of unknown function or antigenicity. To date 40 different genotypes of TVT have been detected. Present study is aimed to determine the antigenicity of ORF2 region of TVT genotype1.
Methods: ORF2 region of TTV genotype1 (JA20 isolate) was amplified using gene specific primers and cloned in pET19b expression vector for protein expression. Expressed protein was confirmed by western blotting using anti-His antibodies against N-terminal 6XHis tag of expressed protein. Antigenicity of ORF2 was determined by western blot, detecting anti-TTV antibodies in 50 human sera of which, 10 were healthy control negative for TTV-DNA, 10 liver disease cases negative for TTV-DNA and 30 liver disease cases that were positive for TTV-DNA.

Results: Protein of ORF2 region was successfully expressed and confirmed by western blot. Anti-TTV antibodies were detected in 25 of 30 (83.3%) cases with liver disease that were positive for TTV-DNA. All 10 cases of liver disease and 10 cases of healthy control, those were negative for TTV-DNA, remains negative for anti-TTV antibodies.

Conclusion: The results indicate that ORF2 protein is immunogenic, showing low detection of IgG. The reason could be that the protein expressed in this study by TTV genopype1 is producing genotype-specific immunity. However, the study indicates that the antibodies produced during TTV infection does not neutralize or cross-reacts. Further investigations are required to determine whether the protein can be used to develop assay for detection of genotype/groupenotypo of TTV-infection.

P70

Clindamycin resistance among Staphylococcus aureus causing skin and ear infections from Chennai, South India

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BMC Infectious Diseases 2012, 12(Suppl 1):P70

Background: Antibiotic resistance in S. aureus is one of the major concerns and the rate of MRSA has dramatically increased in recent years. Clindamycin belongs to the MLS group and is used to treat skin and soft tissue infections. Resistance to clindamycin may be inducible (IMLSB) or constitutive (cMLSB) and it is present both in MRSA and MSSA. Treatment failure has been reported both in IMLSB and cMLSB cases. Knowledge on clindamycin resistance is important for its proper use. Hence, the present study was done to detect clindamycin resistance among S. aureus causing skin and ear infections.

Methods: 84 samples (skin – 55, ear discharge – 29) were collected from the OPD and wards of tertiary hospital in Chennai. Isolation and identification of S. aureus was done according to standard protocol. Antibiotic susceptibility pattern was tested for all isolates to various antibiotics. MRSA screening was done by cefoxitin disc diffusion method. Erythromycin induced clindamycin resistance was detected by using D-test. MIC was determined by agar dilution method.

Results: From the 84 clinical samples, 49 S. aureus isolates were obtained of which 32 (65%) were MRSA. 32 (65%) strains were resistant to erythromycin and 8 (6.1%) showed resistance to clindamycin with MIC of 256 μg/mL. Of the 32 erythromycin resistant S. aureus isolates, 18 exhibited inducible clindamycin resistance. All clindamycin resistant isolates were found to be MRSA.

Conclusion: Increasing resistance to clindamycin has been found among MRSA indicating urgent need for guidelines on its proper use to decrease the morbidity and mortality by MRSA infections.
P73 Comparison of sodA and 16S rDNA sequencing for accurate species-level identification of viridans group streptococci (VGS) isolated from patients with infective endocarditis

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Background: Viridans group streptococci (VGS) are commensal flora of the upper respiratory tract in humans and can cause serious infections, like infective endocarditis, septicaemia, and meningitis. Accurate identification of VGS to the species level is difficult because they share many physiological characteristics. We report a study using sequencing of 16S ribosomal DNA (rDNA) gene and sodA gene to discriminate VGS up to species level.

Materials and methods: Forty-eight strains of VGS isolated from blood cultures of patients with IE were speciated using 16S rDNA and sodA gene sequencing.

Results: The isolates were biochemically identified as mitis group (36), salivarius group (9), mutans group (1), and anginosus group (1) and one unidentified species. Based on 16S rDNA sequencing, the strains were identified as S. sanguinis (10), S. oralis (9), S. mitis (7), S. gordonii (6), S. mitis/oralis (4), S. parasanguinis (3), S. sanguinis/oralis (3), S. sanguinis/mitis (1), and one each of S. mutans and S. anginosus. 27 strains were identified as S. oralis by sodA sequencing which included all the seven S. mitis strains, seven strains which gave ambiguous results and three strains which were not identified by 16S rDNA sequencing. Other species such as S. sanguinis, S. gordonii, S. parasanguinis, S. mutans and S. anginosus were identified by both 16S rDNA and sodA genes.

Conclusion: Identification of VGS upto species level is difficult using phenotypic characteristics alone. 16S rDNA sequence analysis was found to be less reliable than sodA sequencing for the identification of closely related species such as S. mitis and S. oralis.

P74 Prevalence of HCV, HBV and HIV infections in patients and staff of haemodialysis unit

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BMC Infectious Diseases 2012, 12(Suppl 1):P74

Objective: HCV, HBV and HIV are most problematic infections in haemodialysis (HD) patients. The cause and source of infection is multiple in HD patients. Blood transfusion, contaminated equipment and patient to patient transmission are the potential source of infection. Our aim is to find out the prevalence of these infections in our HD centre.

Materials and methods: This study was carried out between June to November 2011. A total of 60 end stage renal disease (ESRD) patients who are on dialysis and 15 technical staff were enrolled in cross sectional study to determine prevalence, risk factor and consequences of HCV infection. Serum samples were tested for HCV, HBV and HIV antibodies using immunochromatographic test. Subsequently anti HCV positive samples analysed with third generation anti HCV test-ELISA.

Results: Prevalence of anti-HCV was 8.33% (5/60) and HBSAg was 1.66% (1/60). All three serological markers were negative in staff and HCV is non reactive in HD patients. HCV infection has correlation with male gender, long term HD and units of blood transfusion. Overt liver disease rarely occurs in the events with ESRD. Chronic liver disease with elevated liver enzymes, were detected in 40% of HCV patients. Level of antibody response was poor in HCV patients range from 1.192-2.064.

Conclusion: The prevalence of HCV and HBV infections are lower in our set up. It can be attributable to undertaking universal precautions, early vaccination, anti viral therapy and isolation of infected patients. It requires stringent adherence to all precautions to decrease the infection rate.

P75 Topoisomerase II isoforms are required for HIV-1 reverse transcription

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BMC Infectious Diseases 2012, 12(Suppl 1):P75

Background: Human topoisomerase II is present in two forms 170KDa α and 180 KDa β isoform. Early reports have indicated that the topolipoisomers and antiviral DNA can block viral replication. The objective of present study is to evaluate specific requirement of topolip isomerases in promoting HIV replication and to characterize the HIV-1 replication intermediates to study molecular action of the topol α or/and β isoforms.

Methods: In topol α or/and β were down regulated by using siRNA mediated gene silencing in SupT1 cells and infected with HIV-1 (93N101) at mentioned time points DNA and RNA were isolated and analyzed by PCR. Co-localization studies were done by using fluorescent antibodies specific to RT, topol α and topol β and images were taken in confocal microscopy.

Results: The results indicated that HIV-1 replication was aborted in topol α or/and β down regulated SupT1 cells. Analysis of the intermediates formed during the HIV-1 replication cycle in topol α-, topol β- and topol α- β-SupT1 cells do not support viral gene expression, integration, PICs formation and cDNA synthesis. Moreover, results revealed the topol α and β co-localization with HIV-1 reverse transcriptase. Taken together, results display the requirement of topoisomerase II isoforms in the event of HIV-1 reverse transcription.

Conclusion: Topoisomerase II isoforms are involved in the HIV-1 lifecycle in the early event of HIV-1 reverse transcription, influencing the phenomenon through an unknown mechanism.

P76 Atypical presentation of opioid withdrawal, an effect of adulteration

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Background: Brown sugar is the impure form of di-Acetylene Morphine with comparable pharmacological effect and withdrawal symptoms. Recent observation regarding the atypicality withdrawal symptomatology in opioid dependants stimulated the present study.

Purpose: To study the abuse pattern and symptom profile in withdrawal state of brown sugar abusers.

Methodology: Consecutive sampling method was used to collect patients with opioid dependence according to DSM IV TR. Abuse pattern was assessed through semi-structure proforma, withdrawal symptoms through clinical opioid withdrawal scale and also chemical analysis of the drug.
Results: Among patients 43.396% had seizure, 26.086% developed confusion after seizure and 17.391% experienced psychotic symptoms. Longer duration and larger quantity of substance abuse leads to higher complications. Seizure episodes occurred between 11 to 92 hrs of last intake with a median of 30 hrs. The seizure frequency had strong correlation with daily doses (r=0.697) and frequency (r=0.527) but is weakly correlated with withdrawal severity (r=0.425). Chemical analysis of illicit drug revealed that caffeine constitutes greater proportion and opioid like substance a minor quantity.

Conclusion: Complications like seizure, delirium and psychosis are common in withdrawal. Complication is higher among high quantity and high frequency users. Delirium and psychosis might be a complication of seizure. Adulteration with toxic substance might be a cause for atypical symptoms which leads to a life threatening condition and warrants preventive cure from such illicit drug as opium substitution therapy.

P77
Scrub typhus-experience from a South Indian tertiary care hospital
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Background: Scrub typhus, an emerging rickettssial disease caused by Orientia tsutsugamushi, is spread by the bite of larval trombiculid mites. The infection manifests as a febrile illness with diverse manifestations. This infection has to be differentiated from other tropical fevers. Our experience of 48 patients with scrub typhus admitted recently to our hospital is discussed.

Methods: Clinical features of 48 patients hospitalized between August and November 2011 with fever, positive OX-K Weil Felix test and clinically diagnosed as scrub typhus were analyzed. Patients with other established causes of fever were excluded.

Results: All these adult patients hailed from the rural Telangana region of Andhra Pradesh. Eighteen out of 48 were farmers. The average duration of fever was 11days. Eschar was noted in only 12.5% patients. Cough and breathlessness occurred in 30% cases. Central nervous system manifestations in the form of drowsiness and seizures were seen in 25% cases. Signs of consolidation were seen in 40% of cases. Thrombocytopenia was seen in 37.5% patients. All patients had elevation of SGOT and SGPT, while 52% patients had elevation of serum alkaline phosphatase. Acute renal failure was seen in 33% patients. 10.4% patients required mechanical ventilation and those in the physical domain were the lowest. Inter-domain consistency and Grocott methamine silver staining (GMS) and nested PCR targeting mitochondrial larger subunit region (mtLSU) was optimized. The optimized PCR was applied on all clinical specimens and amplified products were subjected to DNA sequencing and the sequencing results were analyzed using BLAST analysis software to deduce the sequence homology.

Results: Of the 104 clinical specimens included in the study, P. jirovecii was detected in 35 sputum specimens (36.4%) by KOH Calcioflour and GMS staining. Nested PCR using primers targeting the mtLSU region was specific and sensitive to detect 500fg of P. jirovecii DNA with base pair product size of 346 for first round and 120bp for the second round. The optimized PCR was applied on all sputum specimens, resulting in 47.84% (46/104) positivity for detecting the genome of P. jirovecii. The DNA sequencing of the amplified PCR products showed 98-100% sequence similarity with P. jirovecii deposited in the genbank.

Conclusions: Nested PCR based DNA sequencing targeting mtLSU region is a sensitive, specific, rapid and reliable laboratory diagnostic tool for the detection of PJP directly from sputum specimens.

P79
Clinico-epidemiological profile and quality of life of PLWHA in Central India
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Background: Advances in drug therapy have dramatically extended the life of people living with HIV/AIDS (PLWHA). Empirical evidence shows that as the HIV disease progresses, quality of life deteriorates. PLWHA face physiological, physical, psychological and socio-cultural problems that are caused by these factors affect quality of life. The purpose of this study was to study the clinico-epidemiological profile and assess quality of life of PLWHA.

Methods: Study involved cross sectional assessment of 754 PLWHA above 18 years of age and having treatment duration ≥ 6 months. Socio-demographic characteristics and clinical profile which included associated morbidities, WHO clinical staging and CD4 counts of the patients were studied. Quality of life was assessed with WHO QOL BREF instrument. Chi-square test, Z-test, Pearson’s correlation coefficient, ANOVA with Bonferroni post-hoc test and Multiple Logistic Regression were used for analysis.

Results: Out of 754 study subjects, 61.01% were males, 67.24% were married and 22.2% patients suffered from peripheral neuropathy. Scores in the independence domain were highest and those in the physical domain were the lowest. Inter-domain consistency was found to be good (p=0.001). Age ≥ 50 years (OR 5.71, p=0.001), female sex (OR 2.1, p=0.010), illiteracy (OR 1.98, p=0.045), no spouse support (OR 3.06, p=0.001) and higher WHO clinical staging (OR 1.3, p=0.042) were found to be the major risk factors for poor quality of life.

Conclusions: Though anti-retroviral therapy has prolonged life of PLWHA, their quality of life remains poor.

P80
Identification of SFPQ as novel interacting partner of HIV-1 Integrase and its functional characterization
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BMC Infectious Diseases 2012, 12(Suppl 1):P80

Background: HIV-1 requires the support of its appropriate host for the survival and propagation like any other parasite. These host cell factors have been reported to be involved in different stages of viral life cycle. The nuclear import and infection maintenance of HIV-1 Integrase (IN) in
human cell is dependent upon the integration efficiency of the proviral DNA and stability of viral RNA. In our study we identified a new host cell interacting factor for HIV-1 IN, SFPQ-a RNA splicing protein, by cross-linking, pull down and mass spectrometry.

Methods: This study involved gene cloning, protein expression, purification, cell culture, transfection and selection of stable cell lines for the HIV-1 IN GFP fusion protein and in-vitro IN activity assays study.

Results: We identified a new host cell interacting factor for HIV-1 IN-SFPQ, an RNA splicing protein that interacts with HIV-1, shows 5 fold binding (Kd 0.05 µM) and modulates its disintegration activity.

Conclusions: This study presents SFPQ as a novel cellular factor which has functional interaction with HIV-1 IN. SFPQ, revealed that this protein is not only recruited to the sites where viral genome integration takes place but also increases the disintegration activity and 3’ end processing activities of HIV-1 IN.

P81

Diagnostic and screening utility of various test methods for malaria – a comparative study of malarial parasites in blood smear, quantitative buffy coat and detection of malarial antigen by immunochromatography and ELISA

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BMC Infectious Diseases 2012, 12(Suppl 1):P81

Background: Malaria causes about 250 million cases of fever and 1 million deaths annually. It is one of the causes of poverty and a major hindrance to economic development. Malaria is caused by protozoa of the genus plasmodium. Early accurate diagnosis is essential to start treatment within 24 hours and to avoid empirical therapy with toxic drugs. This study is aimed at finding out the best diagnostic and screening method for malaria.

Methods: Blood from 200 clinically suspected malaria patients were subjected to peripheral blood smear examination, quantitative buffy coat, immunochromatography and enzyme linked immunosorbent assay. The sensitivity and specificity were analyzed. The duration, equipment, expertise, electricity and economy needed were considered.

Results: Peripheral blood smear is highly reliable, species specific, requires expertise and is time consuming. Quantitative buffy coat is a reliable rapid test, but needs economy and experts. Immunochromatography is a rapid, economical, low sensitivity method. Enzyme linked immunosorbant assay is a economical, time consuming, low sensitivity method.

Conclusion: Peripheral blood smear remains the gold standard technique while quantitative buffy coat is the most suitable rapid test in well equipped laboratories and blood banks with heavy work load. Immunochromatography and enzyme linked immunosorbent assay are suitable for population screening in endemic areas and to monitor anti-malarial treatment.

P82

Coupled jumping frogs/particle swarm optimization for estimating the parameters of three dimensional HIV model

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BMC Infectious Diseases 2012, 12(Suppl 1):P82

Background: The three dimensional model of HIV/AIDS is the basis of active research since it includes most aspects regarding the interaction of HIV with patient’s immune system. This model is parameterized by six parameters namely the death rate of CD4 cells, rate of infection of CD4 cells by virus, death rate of CD8 cells, rate of increase of CD8 cells in response to increased viral load, rate of increase of viral load, and rate of decrease of viral load respectively. The objective of this work is to efficiently estimate the HIV parameters using available measurements of viral load.

Methods: The HIV parameters were estimated using coupled jumping frogs/particle swarm optimization technique and viral load measurements. A total number of 25 particles and 25 frogs were used for estimation. The algorithm was run for 20 iterations. The performance of the proposed method for estimation of HIV parameters was assessed in terms of percentage accuracy and estimation time.

Results: The percentage accuracy in estimation of HIV parameters a, b, c, d, e and f was found to be 93.30%, 92.40%, 94.04%, 93.01%, 100% and 98.41% respectively. The total time for estimation was observed to be 426 seconds. Further, the minimum estimation error was achieved within 12 iterations.

Conclusion: For using HIV model for treatment planning, the model parameters must be estimated from measurements acquired on equipment which is accessible to local health services. Results demonstrate that the proposed method is efficient for estimation of all the six parameters of the HIV model.
L89M (74.28%) positions in both group of patients while other mutations were at positions 35, 37, 45, 60, 62, 77, and 82 in few cases. Interestingly, one first line drug experienced patient showed major DR mutations at D30N and M46I positions. Majority (94.28%) was belonging to subtype C and 2 patients were belonging to subtypes A (A1).

Conclusion: HIV-1 subtype C predominates in northern India followed by subtype A. Major DR mutation M46I are suggested to confer low levels of resistance to ATV, FPV, IDV, LPV, NFV and TPV-D30N confers resistance only to NFV. Resistance testing in HIV-1 infected patients should be performed before the initiation of therapy for better therapeutic outcome.

P85
Antitubercular drugs induced hepatic oxidative stress and ultrastructural changes in rats
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Background: As the adverse side effect hepatotoxicity, constitutes an essential part of antituberculosis chemotherapy, the present study was designed to investigate the pathogenesis of antituberculosis (anti-TB) drugs induced hepatic effects in rats with the first-line treatment regimen of isoniazid, rifampicin and pyrazinamide.

Methods: The rats were divided into three groups (n=6 per group), group I served as a control, group II received orally combination of isoniazid (15mg/kg body weight), rifampicin (20mg/kg body weight) and pyrazinamide (35mg/kg body weight) daily for 45 days and group III received simultaneously Silymarin (50mg/kg body weight) and combination of anti-TB drugs at the above mentioned dosages for 45 days. After the experimental period, the levels of malondialdehyde (MDA, oxidative stress marker) and lipid profile was evaluated in serum. The data were analyzed by Duncan’s multiple range tests. The pathological and morphological changes were examined histologically and electron microscopically.

Results: The rats administered anti-TB drugs alone, showed a significantly increase in serum MDA levels and lipid profile (p<0.001). Histopathological features of group II rats showed inflammatory cell infiltration and spotty necrosis. The electron micrograph results indicated kupffer cell hyperplasia, swollen mitochondria and loss of cell architecture. Co-administration of Silymarin significantly decreased anti-TB drugs-induced changes in serum MDA levels, lipids (p<0.001) and retained the liver integrity.

Conclusions: The anti-TB drugs can induce hepatic oxidative stress and the level of serum MDA may be a more sensitive biomarker for monitoring drug-induced hepatotoxicity. Hepatoprotective compounds with antioxidants potential can be supplemented to prevent anti-TB drugs induced cellular oxidative stress.

P86
Studies on the extended spectrum beta lactamases activity in isolates from diabetes patients
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BMC Infectious Diseases 2012, 12(Suppl 1):P86

Background: Extended spectrum beta-lactamases (ESBLs) are enzymes produced by some gram negative bacilli that mediate resistance to extended-spectrum cephalosporins and aztreonam. ESBLs are commonly recognized in a variety of Enterobacteriaceae and Pseudomonas aeruginosa isolates. This study focused on the prevalence of ESBL producing strains from diabetes patients and their antimicrobial correlation.

Methods: ESBL activity studied in different gram negative bacteria isolated from x33 urine samples were subjected to antimicrobial susceptibility testing by Kirby-Bauer method as per CLSI guidelines antimicrobials agents (Cefpodoxime, Cefazidime, Cefotaxime, & Ceftriaxone) selected for testing along with combinations of antimicrobials Cefoprazone sublactam (CFS), Piperacillin-tazobactam (PT) Amoxiclav (AC) were compared for their ability to detect ESBL producers phenotypically.

Results: Among 4446 samples processed 3426 showed ESBL activity in different gram negative bacilli 85% in E.coli, 7% Klebsiella pneumoniae, 0.5% Pseudomonas aeruginosa, 0.5% Citrobacter koseri, 1% in Proteus mirabilis.

Conclusion: There is a significant increase in the prevalence of ESBL Ecoli when compared to other gram negative isolates. Detection of ESBL by phenotypic method in the absence of molecular testing is considered as timely, affordable and appropriate measure in deciding the antibiotic therapy.

P87
Cross-clade neutralization potential of the plasma of antiretroviral naive HIV-1 infected children from north India
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BMC Infectious Diseases 2012, 12(Suppl 1):P87

Background: In this cross-sectional study, we evaluated the efficiency of the plasma of HIV-1 infected children from north India against a standard panel of pseudoviruses.

Methods: We recruited 38 antiretroviral naive HIV-1 infected children after getting written informed consent from their parents/guardians. The study was approved by the institute ethics committee. Neutralization efficiency of the patients’ plasma was tested against tier 2 pseudoviruses (3 clade C - ZM53, Du172.17 and Du156.12 and 3 clade B - RHPA4259.7, TRO.11 and SC422661.8) obtained from NIH AIDS Research and Reference Reagent Program by TZM-bl assay. The inhibitory dilution at 50% neutralization (ID50 titers) was determined by non-linear regression by the method of least squares. Correlation tests were carried out using Spearman rank correlation test.

Results: The median age of the children was six years (range 1.5-14). The median viral load was 24000 RNA copies/ml (range <47-585000) and CD4 count was 655 cells/µl (range 131-2458). Cross neutralization was observed in 28.9% (11/38) of the children. Clade specific neutralization was observed in 47.4% (18/38) against clade C and 7.9% (3/38) against clade B while 15.8% (6/38) of the children did not show neutralization against any of the viruses. There was a significant positive correlation between viremia and neutralization efficiency against two of the viruses studied (Du172 r=0.49; p=0.007 and RHPA r=0.47; p=0.01).

Conclusion: This is the first report on the neutralization efficiency of the plasma of HIV-1 infected Indian children against tier 2 pseudoviruses. Cross-clade neutralizing antibodies were observed in 29% of them.

P88
Defective maturation of dendritic cells during HIV-1 infection is associated with increased expression of SOCS-1
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BMC Infectious Diseases 2012, 12(Suppl 1):P88

Background: During chronic HIV-1 infection, upregulation in the expression of certain negative regulatory factors has been implicated recently as a cause of defects in dendritic cells (DCs). We aim to study the association of one such factor, the suppressor of cytokine signaling-1 (SOCS-1) gene with DC dysfunction during HIV-1 infection.

Methods: DCs from 21 therapy naive (mean CD4: 256 cells/mm³), 21 patients on anti-retrotherapy (mean CD4: 342 cells/mm³) and 14 healthy controls were immunophenotyped for maturation markers at baseline and after 5 hour ex vivo stimulation with TLR-4 ligand, LPS, by flowcytometry. Subsequently, the expression of SOCS-1 gene and the cytokine levels were assessed in monocyte-derived DCs (Mo-DC) of healthy donors exposed to LPS and HIV-1 gp120 by real time PCR and flowcytometry respectively.
Results: The myeloid DCs of untreated subjects had significantly lower responsiveness to LPS stimulation as indicated by lower upregulation of CD83 (mean±SE: 31±4.4 vs. 50±3) and CD80 (30±4 vs. 40±3) as compared to healthy controls. Treated patients had a higher upregulation of CD83 (mean±SE: 38±9) and CD80 (mean±SE: 33±3) though not significantly higher than untreated patients. The expression of SOCS-1 was higher upon exposure to HIV-1 gp120 than in LPS in 5 healthy controls assessed and their culture supernatants showed decreased levels of all the cytokines, mainly IL-6 and TNF-α.

Conclusions: Therapy naive patients exhibit deficient DC maturation upon LPS stimulation, which is partially restored following antiretroviral treatment. An increased expression of SOCS-1 gene upon gp120 exposure suggests a possible role of SOCS-1 in DC impairment.

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**P90**

Improving blood safety using fourth generation HIV ELISA as the screening tool in blood banks – an Indian experience

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**Background:** The newer fourth generation HIV ELISA has been shown to have an increased sensitivity compared to third generation ELISA.

**Objectives:** To estimate the yield of fourth generation HIV ELISA compared to third generation HIV ELISA assay and to determine HIV seroprevalence among blood donors.

**Materials and methods:** This prospective study involved 10200 blood donors – 6800 voluntary donors (3400 – students and 3400 – non students) and 3400 replacement donors. All blood units were tested using third and fourth generation ELISA. All positive & borderline positive samples were confirmed by Western Blot (WB).

**Results:** The HIV seroprevalence was estimated to be 1.37/1000 donations with 3rd generation and 3.62/1000 donations with 4th generation ELISA (p>0.05). Of the 17 samples which were 4th generation ELISA positive and WB positive, 11 were positive with 3rd generation ELISA. Fourth generation ELISA tested 19 additional samples positive and 7 samples as possibly positive which were tested negative with 3rd generation ELISA, giving an additional yield of 26 window period units per 10200 donations i.e. 2.5/1000 donations. Of these, 6 were WB positive giving a yield of 0.58 window period units per 1000 donations. Since 2-3 components are prepared from each blood unit, the yield can be increased to 12-18/10200 donations. With an annual donation of nearly 46000 blood units, this will be 54-81 positive donations.

**Conclusions:** Prevention of 54-81 individuals from acquiring new transfusion transmitted HIV infection with the newer fourth generation HIV ELISA assay with better performance and comparable cost is a cost-effective strategy.

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**P91**

Emerging healthcare issues and needs of young MSM & TG in India: needs assessment from multi-stakeholders perspective

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**Background:** There is inadequate research in India on young MSM and TGs and not much multi-systemic insight is taken into healthcare decisions. Hence, healthcare needs and issues of young MSM & TGs need to be studied from a multi-stakeholder perspective.

**Methods:** An online pilot survey containing a self-administered 14 item questionnaire exploring perception of problems, needs and associated factors was conducted. 52 participants took part in this survey. Data was analyzed using simple statistics, and qualitative responses were thematically analyzed using QDA.

**Results:** a) Problems: young MSMs & TGs engage in unprotected anal intercourse (80.9%), risk sex with people unknown of their HIV status (66%), drug and tobacco (59.6%) and alcohol consumption (55.3%). All participants perceived that MSM & TGs don’t use condoms consistently. They also experience mental health problems like anxiety and depression (80.9%). b) Associated factors: QDA outlined following major factors for engagement with health risk behaviours: i) criminalization of homosexuality; ii) social alienation, lack of social/familial support and poor acceptance; iii) psychological trauma, loneliness; iv) lack of knowledge about safe sex & STDs; v) problems forming a coherent self-identity; vi) fear of losing partner and inability to maintain stable relationship; vii) condoms as barriers in pleasure and engagement for thrill and viii) poor self-esteem and peer-pressure in the face of ix) financial needs.

**Conclusion:** Healthcare needs and issues of young MSM & TG needs to be emphasized with more comprehensive understanding of their social positioning and psycho-social needs than a mere emphasis on HIV/AIDS.

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**P92**

Efficacy of anti-diarrheal activity of *Pedalium murex* L., in wistar albino rats

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**Background:** Diarrhea is a major health problem especially for children under the age of 5 and up to 17% of children admitted in the pediatric ward die of diarrhea. A range of medicinal plants with anti-diarrheal properties is widely used by traditional healers.

**Methods:** The ethyl acetate extract of *Pedalium murex* L. (250, 500, and 1000 mg/kg body weight) was administered orally to three groups of rats (five animals per group) in order to evaluate the extract against castor oil-induced diarrhea model in rat. Two other groups received normal saline (5 mg/kg) and loperamide (5 mg/kg) as positive control.

The effect of the extract on intestinal transit and castor oil-induced intestinal fluid accumulation (enteropooling) was assessed.

**Results:** At oral doses of 250, 500, and 1000 mg/kg body weight, the plant extract showed pronounced and dose-dependent anti-diarrheal activity. The protective role of the extract at 1000 mg/kg was comparable to that of the reference drug, loperamide (5mg/kg). The extract (1000 mg/kg) produced a decrease in intestinal transit comparable to atropine (5mg/kg), and significantly (p<0.01) inhibited castor oil-induced enteropooling. No mortality and visible signs of general weakness were observed in the rats following the extract administration of up to a dose of 6000 mg/kg.

**Conclusion:** The results showed that the extract of *Pedalium murex* L. has a significant anti-diarrheal activity which supports its use in traditional herbal medicine practice.

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**P93**

Understanding dengue transmission by using participatory research and community-focused strategies for prevention and control in Bangladesh

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**Background:** Globally, dengue has become one of the most alarming infectious diseases and its resurgence reflects the failure of traditional reductionistic disciplinary approach. Because neither an effective vaccine nor an effective vector control program is available for dengue prevention and control, a Community-Based Approach (CBA) to vector control and individual behavior change has been implemented in many countries. However, by and large, the CBA has failed as it ignored local community members’ basic needs and perspectives in these processes. By challenging reductionist notions, this research will delineate the mental maps of local urban residents of the City of Dhaka, Bangladesh concerning dengue transmission and methods of dengue prevention and control.
Methods: This study involved focus group discussion in 3 wards of Dhaka City Corporation, semi-structured interview of 30 stakeholders representatives; 900 ward/community members (300 from each ward); 18 policy- and/or decision makers (national and local institutions) and community members’ mental map construction of 24 ward representatives (supplemented by 300 ward members).

Results: This study revealed the lack of intersectoral coordination between local and national institutions dealing with disease and household sanitation, and highlights the difficulties in avoiding dung vectors in urban areas with irregular water supply, poor sanitation services, and finally the location of large and small construction zones all over the city.

Conclusion: The conclusion emphasizes the importance of the knowledge about the daily problems faced by the community members and partnership needed in all sectors to address water supply problem and disease surveillance systems.

Background: Researchers have reported that 9400 HIV cases were detected in the voluntary screening of drug addicts in Harm Reduction Programmes, and screening in prison and Narcotics Rehabilitation Centers in 2011. However, dental treatment for HIV patients is highly costly by high uncertainty resulting from the changes of epidemic profile after receiving medical treatment, relative inadequacy of dental treatment and rules and regulations. Thus institutional HIV dental care cost could pose challenges to government healthcare expenditure.

Methods: The marginal cost for dental treatment is estimated based on the case scenario of prison X in Malaysia. Based on literature review and economic reasoning, an integrated cost planning model is formed to address the current needs in cost planning.

Results: The marginal cost for prison dental treatment is estimated to increase by at least 3 folds for prevalence cases and at least 7 folds for surveillance case in short run. The cost pyramid is formed with the base of surveillance cases and 1.7% of Salmonella contamination was not detected.

Biochemical mechanism of clinical resistance to rilpivirine

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BMC Infectious Diseases 2012, 12(Suppl 1):P94

Background: The introduction of HAART has significantly prolonged the life span of HIV-infected patients. However, the error-prone nature of HIV-1 reverse transcriptase (HIV-1 RT) results in the emergence of drug-resistant viruses and threatens the effectiveness of HAART. HIV-1 RT is a primary target of two classes of drugs: nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Recent phase III clinical trials have shown that two HIV-1 RT mutations, E138K and M184I, were the most frequent mutations found in patients that experienced virological failure during therapy that included rilpivirine (RPV), emtricitabine (FTC), and tenofovir (TDF).

Methods: To investigate the mechanistic basis for resistance caused by the E138K and M184I mutations we used transient kinetics to characterize the enzymatic properties and drug susceptibility of RTs with these mutations and determined the biochemical mechanism of resistance to RPV. Specifically, we compared wild-type (WT) RT to RTs mutated in one or both of the enzymatic subunits: p66M184I/p51M184I, p66E138K/p51E138K, p66E138K/p51M184I, p66E138K/p51WT, p66WT/p51138K.

Results: Our results show that M184I reduces the catalytic efficiency of RT by more than two-fold (p66M184I/p51M184I has more than 2-fold reduced kpol/Kd.dNTP with respect to WT). This defect is compensated by mutation E138K either in both subunits or only in p51 subunit (p66WT/p51138K).

Conclusion: None of the mutations affected the template-primer binding affinity of RT. As expected, M184I does not reduce the susceptibility to RPV. Instead, RPV resistance is achieved by reduction in the binding affinity of the drug to RT because of the E138K mutation in the p51 subunit.

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Background: Food-borne illnesses have high prevalence in the world, which are caused by consumption of water or food contaminated. Nowadays, involvement caused by Salmonella bacterium, serotype enteritidis and typhimurium are the major infection all around the world. So the purpose of this study is detection of Salmonella enteritidis, typhi and typhimurium in different kinds of foods by multiplex PCR method.

Methods: The study was conducted on 170 samples of food products including, milk, beef, poultry, salad dressing and 80 samples in contact with food items such as knives, cutting boards and hands of personnel working in a hospital's kitchen. After collecting the samples, the standard diagnostic method for detection of bacteria were performed and after extracting the DNA, the multiplex PCR method has been used for determination of Salmonella serotypes.

Results: From all of the tested food samples (170), 1.7% of Salmonella contamination has observed that 1.1% related to typhimurium and 0.6% related to Salmonella enteritidis that had separated from beef, and during the tests conducted on samples that were in contact with food items, Salmonella contamination was not detected.

Conclusion: Results indicated that, quality control of food products in processing and production stages observing hygiene issues are critically important in preventing food-borne diseases. And also the methods like molecular diagnostic such as multiplex PCR besides the cultivation of bacteria and other microbes can be helpful in diagnostic confirmation.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Nosraty et al. Detection of Salmonella enteritidis, typhi and typhimurium in foods by multiplex PCR in children hospital. BMC Infectious Diseases 2012, 12(Suppl 1):P95