ICIDN-2015 (December 15-18, 2015), Kathmandu, Nepal

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Kathmandu, Nepal

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December 15-18, 2015 Kathmandu, Nepal

2nd International Conference on Infectious Diseases and Nanomedicine-2015

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WELCOME

Respected Distinguished Guests, Colleagues and Friends,

It is our great pleasure to welcome you in the *2nd International Conference on Infectious Diseases and Nanomedicine (ICIDN 2015)* from Dec. 15 to 18, 2015 which is being jointly organized by Nepalese Forum for Medical Microbiology (NFMM), Nepal Polymer Institute (NPI) and CAS-TWAS Centre of Excellence for Biotechnology (CoEBio), Institute of Microbiology, Chinese Academy of Sciences (CAS) in collaboration with Kathmandu University, Dhulikhel, Kavre, Nepal and Nepal Academy of Science and Technology (NAST) with support from American Society for Microbiology (ASM), Washington DC, USA and European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Basel, Switzerland at Park Village Resort/Hotel Budhanilkantha, Kathmandu. The congress will be attended by more than 200 participants including renowned scientists from 18 countries from across the globe.

The first edition of this tri-annual series of ICIDN held in Kathmandu from Dec. 15 to 18, 2012 was highly successful in bringing together more than 180 participants from 21 countries. The ICIDN-2015 focuses on *Molecular microbiology of infectious diseases and potential applications of nanotechnology for their diagnosis and treatments*. This in turn will provide the unique opportunity for presentation and sharing of innovations of microbiologists, immunologists, molecular biologists, epidemiologists, pathologists, chemists, pharmacists, polymer/biomedical engineers, material scientists, biotechnologists, nanotechnologists, clinicians, public health experts, and other biomedical scientists from both the academia and industries. Emerging infectious diseases, antimicrobial resistance, drug design, and drug delivery will form an integral part of the ICIDN-2015 endeavors. A pre-conference workshop arranged on the related topic will also provide first-hand information to the students and early career scientists. The conference and accompanying workshop are hoped to be a milestone in promoting interdisciplinary education and researches in the field of microbiology, infectious diseases, material sciences and nanotechnology in Nepal and the region.

We extend our warm welcome to all our ICIDN – 2015 delegates and wish a pleasant stay in Kathmandu as well as fruitful discussions and new networking. Kathmandu valley offers plenty of cultural heritage and ancient architectures. We request of foreign delegates and to spare some time to visit our cities and enjoy the natural beauty.

We sincerely thank all our well-wishers and supporters, who have contributed to the ICIDN – 2015 in various ways.

Rameshwar Adhikari

On behalf of organizing committee of ICIDN 2015

Opening Lectures

Opening Lecture (OL) -1

Cancer and Infectious Diseases: Bacterial Proteins/Peptides for Therapy

Ananda M. Chakrabarty

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Bacteria are the causative agents of many infectious diseases and bacteria such as *Helicobacter pylori* are known to cause gastric cancer in humans and animals while *Agrobacterium tumefaciens* is known to trigger crown gall in plants. Unlike such cancer-causing bacteria, however, many pathogenic bacteria have been known for over hundred years to fight cancer, allowing tumor regression in cancer patients. An example of such cancer-fighting bacteria will be the opportunistic pathogen *Pseudomonas aeruginosa* that can infect the lungs of cystic fibrosis patients forming long-lasting biofilms which are hard to eradicate. It has been postulated that biofilm-forming bacteria with long term residence in the human body consider the human body as their habitat and become protective of it by fighting outside invaders such as cancers, viruses and parasites. Indeed, *Pseudomonas aeruginosa* is known to secrete a protein known as azurin on contact with cancer cells. Azurin is known to enter preferentially to cancer cells, but less so to normal cells, and kill the cancer cells through multiple mechanisms to prevent resistance development. Azurin has also been shown to be effective in preventing host cell infection by viruses such as HIV-1 and parasites such as *Plasmodium falciparum*. A 28 amino acid fragment of azurin, termed p28, has completed two phase I trials in the United States, exhibiting very little toxicity but significant beneficial effect including partial and complete regression of tumors in both stage IV adult and pediatric brain tumor patients (www.cdgti.com). Another 20 amino acid long peptide, termed AT-01C, from *Mycobacterium bovis* protein MPT63 has been shown to exhibit strong anticancer activity against a range of cancers (www.amritatherapeutics.com).

Opening Lecture (OL) -2

Carbon Nanotube and Its Medical Applications

Mushahid Husain

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Carbon nanotube (CNT) is an extraordinary material which shows extraordinary mechanical, thermal and electrical properties. These remarkable properties and the high surface area make CNTs suitable for various applications. Due to their high surface area, excellent chemical stability, and rich electronic polyaromatic structure, CNTs have been extensively studied for its use in pharmacy and medicine, since the beginning of the 21st century. CNTs are able to absorb or conjugate with a wide variety of therapeutic molecules such as drugs, proteins, antibodies, DNA, enzymes, etc. They have been proven to be an admirable carrier for drug delivery by penetrating into the cells directly and keeping the drug intact without metabolism during transport in the body. Many researchers have shown that when bonded to CNTs, these molecules are transported more efficiently and safely into cells than by conventional methods. CNTs have also been examined for gene therapy, immunotherapy, tissue regeneration and diagnosis of different elements. Therefore, in a very short time, CNTs have become the focus of attention by various researchers in an extensive variety of disciplines. They may be promising antioxidants for health protective effect and ailment prevention in the future.

We have synthesized CNTs particularly SWCNTs of specific diameters vary from 1 to 4 nm using plasma enhanced chemical vapor deposition (PECVD) method suitable for drug delivery as well as for drug analysis. PECVD method favours low temperature synthesis of VA-CNTs. The SWCNTs grown samples were characterized by various techniques including field emission Scanning Electron Microscope (FESEM), Raman spectroscope and High Resolution Transmission Electron Microscope (HRTEM). The grown SWCNTs were also studied for sensing applications of various atmospheric pollutants including NH_3 and NO_2. The results show that the grown SWCNTs are good for sensing applications and particularly for biosensing applications. In the present talk, the applications of CNTs to get better MRI pictures, genes therapy, bone treatments and cancer treatment, will be discussed.

Keynote Lectures

Keynote Lecture (KL) -1

How does vaccinia virus interfere with interferon? New findings with an old vaccine

Geoffrey L Smith

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Vaccinia virus (VACV) is the vaccine that was used to eradicate smallpox and is the most extensively characterised poxvirus. Like other poxviruses, VACV replicates in the cytoplasm and has a large, complex virus particle and a linear, double stranded DNA genome. The VACV genome encodes about 200 genes and is arranged with genes encoding proteins that are essential for virus replication present in the central half of the genome. These genes are highly conserved between different VACV strains and orthopoxviruses. In contrast, genes located towards the ends of the genome are more variable between these viruses and encode proteins that are non-essential for virus replication but help the virus evade the host innate immune response to infection and influence virulence. This talk will consider this class of gene and especially those that encode proteins that interfere with interferon.

Most mammalian viruses encode at least one protein that helps the virus to evade or interfere with interferon and VACV is no exception and encodes many proteins that do this. These proteins are diverse and function in different ways and at different locations. One group functions within the cytoplasm to block the signalling events induced by the recognition of pathogen associated molecular patters by cellular pattern recognition receptors that would lead to the production and release of interferons. A second group of proteins are secreted outside the cell and function to bind type I or type II interferons extracellularly to prevent these interferons reaching their natural receptors on cells. A third group of VACV proteins function within the infected cell to block the signalling events that interferons induce and thereby block the expression of interferon-stimulated genes. Lastly, additional VACV proteins inhibit the activation or function of interferon-induced proteins that provide protection against subsequent virus infection.

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Keynote Lecture (KL) -2

Development of cancer sensing nanodevices using metamaterial nanostructures

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We study new types of the sensing cancers devices fabricated from quantum dots doped in a metamaterial heterostructure. Metamaterials are a new class of artificial materials with having unique types of optical properties due to their nanoscale organization of their unit cell components [-4]. Their unique electromagnetic properties are different than that of materials found in nature. For example metamaterials possess simultaneously negative effective dielectric permittivity and magnetic permeability for a range of frequencies in the electromagnetic spectrum. This effect is not found in natural materials such as silicon and gold. Recently, metamaterials based on periodic arrangements of metallic nanocomposites have received special attention. It is well known that the metals have negative electric permittivity which leads to the formation of surface plasmon which may generate exceptionally strong localized electromagnetic fields. Heterostructures studies here are formed by fabricating a split-ring resonator and metallic rod metamaterial on a dielectric substrate. An ensemble of non-interacting quantum dots (QDs) are doped near the interface in the heterostructure. The QDs interact with surface plasmon polaritons of the heterostructure. We have calculated the energy exchange between the QDs and the metamaterial in the presence of exciton-surface plasmon interaction. It is found energy transfer is enhanced in the presence of the metamaterial when the exciton and surface plasmon frequencies are resonant. The energy transfer can be switched on and off by applying the external fields such as lasers or stress fields. The present results can be used to make new types of nanoscale cancer detection devices based on metamaterials.

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Keynote Lecture (KL) -3

Significance of Vi Negative Isolates of *Salmonella enterica* Serovar Typhi in causing Typhoid Fever

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Typhoid is a major bacterial disease affecting 33 million people globally each year. Traditionally, pathogenicity of typhoid bacillus has been attributed to Vi (Virulence) capsular antigen. However, we in collaboration with Imperial College, London reported that Vi negative isolates occur naturally and can cause disease. They may make up to 25% of all isolates in some areas. These findings were subsequently substantiated by reports from Nepal and India. It has been found that Vi antigen is not required for causing disease. These findings have completely changed the perception of typhoid vaccine because all current vaccines are based on Vi capsule which are ineffective against Vi negative isolates. This provides selective advantage and they may emerge as a major global threat. We developed diagnostic tools for rapidly differentiating between these isolates. We have focused on establishing conjugate vaccine candidates based on LPS of outer membrane which are present in all *S*. Typhi isolates whether they are Vi +ve or Vi –ve, and can be used universally. We have also focused on structural differences among these variants by using techniques such as GC-MS and NMR.

Keynote Lecture (KL)-4

Role of Molecular Tests in Laboratory Diagnosis of Syphilis

Muhammad Morshed [1, 2]

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B.C. Public Health Microbiology and Reference Laboratory, British Columbia, Vancouver, Canada.

Syphilis is a century old disease with serology being the main method used for diagnosing this disease. Observing syphilis spirochetes through darkfield microscopy has been known to have limitations due to the lack of sensitivity in microscopy and the subjectivity of the morphological state of the spirochete to the technologist. Conversely, molecular tests such as PCR have been known to have great specificity and sensitivity and can detect down to a single spirochete. As microscopy and PCR vary vastly in sensitivity, literatures on the performance of molecular tests were investigated. Comparison of author's own laboratory data on darkfield microscopy and PCR were compared against a "gold standard." This "gold standard" was defined by comparing the cumulative results of two or more test platforms (darkfield microscopy, PCR, real-time PCR, and combine treponemal and non-treponemal serology). Sensitivity and specificity varied according to samples types. However, PCR had higher sensitivity and specificity when compared with darkfield. Further, when darkfield was compared to our established gold standard, the sensitivity and specificity were 58.1% and 98.5% respectively. The observed lower sensitivity is expected due to the limitations of microscopy. When comparing PCR methods to the established gold standard, the sensitivity and specificity were 98.8% and 98.7%. The seemingly lower specificity of PCR compared to the gold standard could be due to the fact that the definition of the gold standards are based on test platforms that are intrinsically lower in sensitivity than compared to PCR. As expected, PCR provided greater sensitivity and specificity than traditional darkfield microscopy. The higher sensitivity observed in PCR has also led to the finding of multiple cases of syphilis infection before standard serological tests. This advantage may prompt physicians to start treatment before serological detection and minimize further transmission. In this presentation, global data on molecular testing will also be discussed.

Invited Lectures

Invited Lecture (IL) -1

Zoonotic Infection in Nepal: Status of Toxoplasmosis

Shiba Kumar Rai

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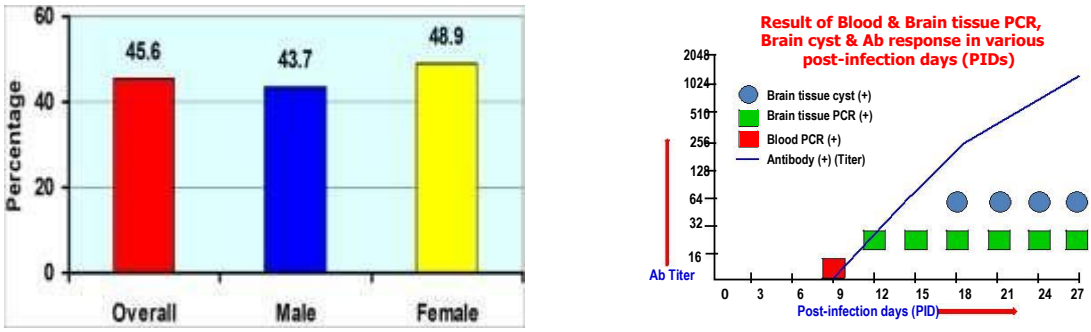
Though non-infectious diseases are on the rise during recent years, infectious diseases still predisposes in the causation of both morbidity and mortality among Nepalese. Of the various types of infections, zoonotic infection alone constitutes one of the major health problems in Nepal. In this paper, status of *Toxoplasma gondii* (protozoa) infection as studied by serological and molecular techniques is described. Of the over 8,000 participants studied, nearly half (45.6%) were infected. Prevalence ranged from 24 to 67% in different geographical areas/study population. Prevalence was higher among females (48.9%) than in males (43.7%) ($p<0.05$). Infection was more common among *Tibeto-Burman* ethnic-group (47%) than in *Indo-Aryans* counterpart (44%). Interestingly, one-third of 20 and less than 20 years population was infected and the infection rate increased with age reaching 67% in elderly population (>60 years) ($P<0.05$). Higher infection rate was seen among pregnant women, women with bad obstetric history and patients with malignancy. A higher *Toxoplasma* IgM antibody positive rate was seen among pregnant women (3%) compared with general population (1%). However, till date, only one case of congenital toxoplasmosis has been reported from Nepal. One of the major source of *Toxoplasma* infection appears to be meat/meat products as indicated by high seroprevalence among common meat animals (chickens: 41%, buffaloes: 63%, sheep/goats: 69% and pigs: 80%) and the higher prevalence among raw meat/meat preparation eating population. Experimental study in mice showed parasitemia only on 9th post infection day (PID) as detected by PCR whereas PCR was positivity in brain tissue was seen on 12th PID and after. *Toxoplasma* antibody became positive on 12th PID and the titer increased steadily thereafter. Brain tissue cyst was detected microscopically only after 18th PID.



(A) (B)

Figure 1. (A) *Toxoplasma* seroprevalence among Nepalese (n >8,000) and (B) Result of experimental study in mice.

Reference

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Invited Lecture (IL)- 2

A randomised controlled trial of gatifloxacin versus ceftriaxone for the treatment of uncomplicated enteric fever in Nepal

Buddha Basnyat

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Third generation cephalosporins and fluoroquinolones are the gold standard enteric (typhoid) fever treatment, but antimicrobial resistance compromises efficacy. We performed an open-label trial, randomising Nepali children and adults to gatifloxacin or ceftriaxone for seven days. The primary outcome was a composite endpoint of treatment failure, defined as: fever for >7 days; or rescue treatment required; or microbiological failure, relapse, or complications until day 28. Secondary outcomes included fever clearance time and faecal carriage. Trial registration: ISRCTN63006567. 239 of 300 intended patients were enrolled before the data safety and monitoring board stopped the trial. In the modified intention-to-treat population, treatment failure occurred in 18/120 (15%) gatifloxacin-treated and 19/119 (16%) ceftriaxone-treated patients (hazard ratio (HR) of time to failure 1·04, 95%CI 0·55-1·98, p=0·91). There was significant heterogeneity (p<0·0001) in the treatment effect between sub-populations: in the blood culture-confirmed population, 16/62 (25%) (gatifloxacin-treated patients failed treatment, compared to 4/54 (7%) ceftriaxone-treated patients (HR 0·24, 95%CI 0·08-0·73, p=0·01). Treatment failure was associated with the emergence of *Salmonella* Typhi exhibiting resistance against fluoroquinolones, requiring the trial to be stopped. In contrast, in culture-negative patients, 2/58 (3%) on gatifloxacin versus 15/65 (23%) on ceftriaxone failed treatment (HR 7·50, 95%CI 1·71-32·80, p=0·01). A similar number of non-severe adverse events occurred in each arm. Fluoroquinolones should no longer be used for enteric fever treatment in Nepal. Additionally, under our study conditions, ceftriaxone is a sub-optimal therapy in a high proportion of patients with culture-negative enteric fever, which further emphasises the need for diagnostic tests for enteric fever and other common febrile disease. Given that antimicrobials, specifically fluoroquinolones, are one of the only routinely used control measures for enteric fever, the assessment of novel diagnostics, new treatment options, and the use of existing vaccines and development of next-generation vaccines are now a greater priority than ever.

Invited Lecture (IL) - 3

Nanoformulations in Neurological Disorders

Bikash Medhi

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Nanoparticles as drug delivery carriers could modernize the treatment for neurodegenerative diseases such as Alzheimer's disease (AD), Autism, Parkinson's disease (PD) and strokes, if applied in the right direction. Muller in 1991, discovered a novel and biocompatible nanocolloidal drug delivery vehicle known as solid lipid nanoparticles (SLNs), which have been recently explored for brain delivery in various neurological disorders like AD, autism etc., and fetched the scientific fraternity with promising results. Galantamine hydrobromide (GH) is a reversible, competitive acetylcholinesterase inhibitor which also acts as an allosterically potentiating ligand for nicotinic acetylcholine, thus exhibiting neuroprotective effects. The issues of lower bioavailability and poor blood brain barrier penetration are the leading factors of compromised efficacy of GH. Recently various studies have been carried out in which both the concerns have been taken care with the help of promising novel carriers, i.e. SLNs. Bioavailability was enhanced by approximately 100% and significant improvement in cognitive deficit was also observed in AD and autism like preclinical protocol. The developed system provides a platform for maximum utilization of present medications by avoiding the adherent hurdles, employing drug delivery approach.

Invited Lecture (IL) -4

Morphology of Nanofibrous Materials for Medicinal Applications

Jakub Širc

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Various medicinal applications such as cell therapy, wound dressings, skin regeneration or corneal transplants require special demands on the structure of used materials. Beside the chemical composition, biocompatibility, permeability and mechanical properties, the morphology is the most important attribute of the constructs. Specific surface area, volume and size of the pores have considerable effect on the cell adhesion, growth and proliferation. In case of the incorporated pharmaceutically active substances their release is also influenced by the internal structure of nanofibers.

In our research projects, nanofibrous synthetic nonwovens were prepared from various polymers by needle-less electrospinning. Scanning electron microscopy was used to observe the samples, to evaluate the fiber diameters and to reveal eventual artefacts in the nanofibrous structure. BET nitrogen adsorption/desorption measurements were employed to measure the specific surface areas. Mercury porosimetry was used to determine total porosities and compare pore size distributions of the prepared samples. Experiments based on the soaking of nanofibers into the non-solving liquid were used to measure total porosities.

Various techniques brought valuable data, however, obtained results show that each method has some disadvantages and limitations. The morphological characterization of nanofibrous materials requires a complex approach and evaluation of the results of various methods.

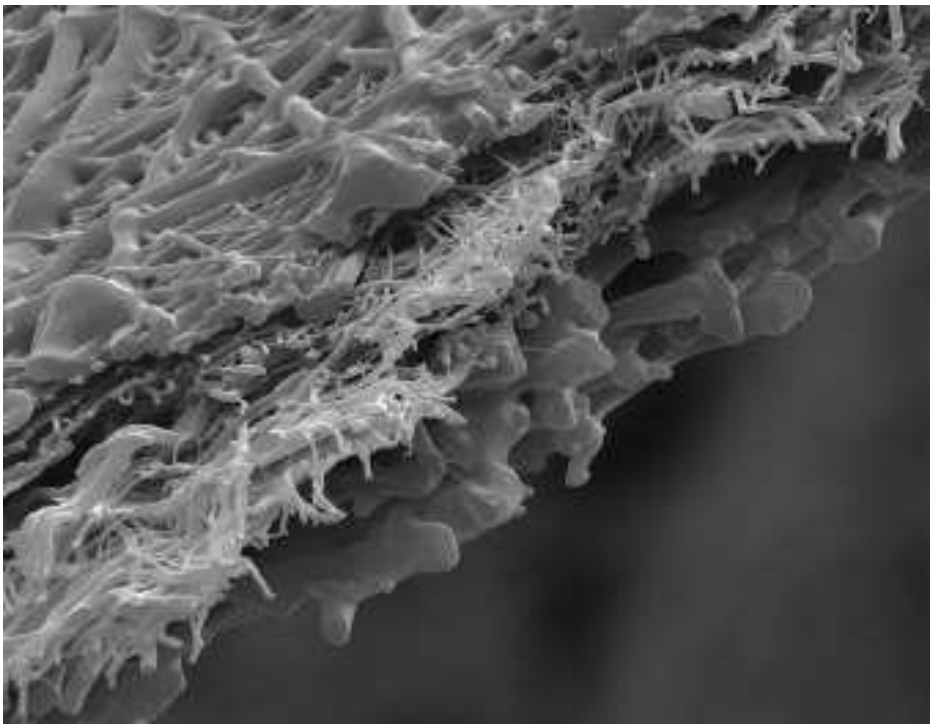

Figure 1. Layer of poly(vinyl alcohol) nanofibers with incorporated broad-spectrum antibiotic Gentamicin, covered by layers of polyurethane nanofibers improving mechanical properties and prolonging antibiotic release.

Invited Lecture (IL) -5

Competitive exclusion of foodborne pathogens by stimulating growth and production of bioactive components of *Lactobacillus casei*

Mengfei Peng, Serajus Salaheen, Debabrata Biswas*

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Lactobacillus casei found in human intestine and mouth is commonly applied for dairy production. Recently, it was found that some byproducts produced by *Lactobacillus* exhibited antimicrobial activities against multiple bacteria. Meanwhile, introduction of prebiotic-like foods (e.g. cocoa) or probiotics or both of them as food supplements in human diets as well as in farm animal feeds is believed to be an effective ways in control/reduce the colonization of foodborne bacterial pathogens infection in the gut environment. We hypothesized that cocoa may stimulate the production antimicrobial components *of Lactobacillus casei* and may potentially inhibit/reduce the colonization and infection of foodborne bacterial pathogens in the gut.

Mixed culture of *L. casei* (LC) with enterohemorrhagic *E. coli* EDL933 (EHEC), *Salmonella enterica* serovar Typhimurium LT2 (ST), or *Listeria monocytogenes* LM2 (LM) showed that LC could competitively exclude (100%) them within 72 h. Further, investigation of cell-free culture supernatant (CFCS) revealed that the antimicrobial effects of LC came from CFCS. CFCS of LC eliminated (100%) EHEC, ST, and LM within 72 h, and 2 h CFCS treatment increased the hydrophobicity of EHEC (5.10 folds), ST (8.48 folds), and LM (2.03 folds). In addition, LC cells exhibited more inhibitive effects than CFCS on cell adhesive and invasive activities of EHEC (52.14% & 90.45%), ST (66.89% & 93.83%), and LM (61.10% & 83.40%). Two clusters of poly-peptides in CFCS were identified by SDS-PAGE, the molecular weights of which are ≈5 KD and 40-45 KD. LC CFCS with overnight growth in the presence of 3% strengthened all of the antimicrobial activities (growth inhibition, outer membrane disruption, and cell infective ability reduction). Liquid chromatography/Mass spectrometry analysis detected 5 unique components in class of flavonoids in LC CFCS with overnight 3% cocoa supplement. Furthermore, qPCR results showed that CFCSs up-regulated the expression level of genes responsible for flagellin synthesis and motility, but down-regulated genes for specific binding and invasion-associated proteins synthesis. The stimulatory effects of cocoa in producing bioactive components of probiotics may aid prevention of foodborne illness caused by major foodborne enteric bacterial pathogens.

Invited Lecture (IL) - 6

Treatment Options in a Post- Antibiotic Era

R. R. Bragg*, van der Westhuizen, W. Coetzee, M., Lee, J-Y., Jansen, A.C., Theron, C., Boucher, C.E.

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It is well known that there are increasing problems with antibiotic resistance, not only in poultry production, but also human medicine. There is increasing pressure to reduce or even totally stop the use of antibiotics in animal production. When this happens, the poultry sector (as well as other animal production systems) will be faced with very serious problems. Our research group is actively investigating various alternatives to treatment of diseases in a post antibiotic era. These alternative treatment options include improved vaccine development, improved biosecurity, and the use of bacteriophages. All three of these treatment options will be briefly reviewed. Our main research emphasis on improved vaccine development revolves around a novel yeast based expression system. Our current research focus on this is the development of a vaccine against avian pathogenic *E. coli*. Improved biosecurity has mainly been focused on the continual use of disinfectants throughout the production cycle in poultry. The use of this system has been shown to significantly reduce bacterial counts and mortalities and has also been shown to reduce the impact of infectious coryza in poultry. If improved biosecurity can reduce mortalities in poultry production, it can also reduce the staggering high numbers of deaths from hospital acquired infections. Our final research area is on the use of bacteriophages, or parts thereof, in the treatment of bacterial diseases. Although phages show much potential, the very high level of specificity could make the use of bacteriophages challenging. The very elaborate defences mechanisms which bacteriophage have to protect them against virus infection will also have a significant impact on the use of bacteriophages to treat bacterial diseases in a post antibiotic era. The use of expressed phage enzymes appears to be a viable alternative to making use of highly specific bacteriophages. The specificity of expressed phage enzymes is currently being tested.

Invited Lecture (IL) -7

Human Rabies in Nepal: a-14-year experience from a tertiary central referral tropical infectious disease hospital

Sher Bahadur Pun

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Rabies is consistently underestimated disease and bears a considerable public health burden in Nepal. There are, however, a very limited number of information on human Rabies in Nepal. The present report attempts to understand animal bites and human rabies trends over a 14-year period (2001-2002 to 2013-2014) in Kathmandu. Data were obtained from the rabies section of the Sukraraj Tropical & Infectious Disease Hospital, then entered into excel spreadsheets, reviewed and analyzed. There was a remarkable increase in the number of animal bites (nearly a 500%) during a 14-year period. Dog bites were the highest (91%) followed by monkey bites (5%) among animal bite cases attended to hospital. Dog bite injuries were more common in children (58%) than in adults. Nearly equal numbers of males and females (1.2:1) were vaccinated with rabies post-exposure prophylaxis following monkey bites. The highest number of rabies cases (16 cases) was reported during 2009-2010. This report provides an insight into the alarming trend of animal bites and human rabies cases in Nepal. Policy makers can use this information to allocate resources properly and to design a more effective rabies elimination strategy in future.

Invited Lecture (IL) -8

Molecular characterization of acquired quinolone resistance in clinical isolates of *Salmonella* Typhi

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Fluoroquinolones are the recommended first-line therapy for the treatment of typhoid fever. The excessive and indiscriminate use of fluoroquinolones has resulted in the emergence of drug-resistant strains leading to numerous outbreaks of the disease. The target of fluoroquinolones is the protein DNA Gyrase and treatment failure is majorly associated with decreased drug susceptibility arising due to mutations in this protein. To explore the molecular basis of induced drug resistance in *Salmonella* Typhi we have analysed the differential behaviour of protein - drug interaction of the fluroquinolones with the wild type and mutant proteins. We estimated the Minimum Inhibitory Concentration (MIC) of commonly used fluoroquinolone representatives from three generations, ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin, for 100 clinical isolates of *Salmonella* Typhi from patients in Indian subcontinent. The MICs were found to be in the range of 0.032 to 8 μg/ml. Subsequent sequencing by PCR of the gene encoding DNA Gyrase revealed point mutations at two specific locations in the quinolone resistance determining region comprising Ser83Phe/Tyr and Asp87Tyr/Gly. The binding capability of fluoroquinolones was computationally analysed in the quinolone binding pocket of wild type protein and four mutants. This was achieved by homology modeling of DNA Gyrase molecule and molecular docking of the drugs individually into the active site of the protein for analysis of their differential binding behaviour. This study has revealed that mutations in DNA Gyrase alter the characteristics of the binding pocket in terms of charge properties, shape and size. This results in loss of crucial molecular interactions present between drug and protein in wild type protein and consequently decreases the binding affinity of fluoroquinolones towards mutant proteins. The present study has resulted in understanding the underlying molecular and structural mechanism for the decrease in fluoroquinolone susceptibility in clinical isolates as a consequence of mutations in DNA Gyrase.

Invited Lecture (IL)- 9

Basanta Pant

Annapurna Neurological Instiute and Allied Sciences, Maitighar, Kathmandu, Nepal

Invited Lecture (IL)- 10

Antifungal Drug Resistance

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Invasive fungal infections have emerged as important causes of morbidity and mortality in immunocompromised patients. Although several antifungal agents have been licensed in recent years,clinical failure due to antifungal drug resistance is becoming a major concern. Understanding the mechanism of resistance and its clinical impact is important, while planning the treatment strategies. Definite progress has been made in the past in understanding the biochemical and molecular basis of the mechanisms contributing to antifungal drug resistance. Quicker and well standardized protocols in determining the sensitivity patterns of clinical isolates of fungi towards the antifungals used in routine practice would help the treating physician in optimizing the therapy. However, unlike determining minimum inhibitory concentrations (MICs) of antibiotics; the estimation of MIC break points in fungi,particularly so in filamentous fungi is complicated and requires standardization of many variables like inoculum size, incubation temperature, incubation time, nature of diluent for antifungal agents, suspending medium for the inoculum, and pH of the medium. Despite the above shortcomings, many laboratories across the world have developed well standardized methods of determining the MICs of clinical isolates of filamentous fungi against various classes of antifungal agents. Over and above, high and low MIC values were correlated with the organisms'phenotypic and molecular pathogenic markers, guiding the clinician with a scope for better patient management. Even then, unresponsiveness to antifungal treatment may be a clinical problem because of a number of agent factors. One of these could be biofilm production by fungi. The exact mechanism contributing to the recalcitrant nature of fungi enclosed inside a biofilm towards antifungal drugs, is being explored. Nevertheless, it becomes a priority and need of the present time to unravel the possibilities of newer antifungal agents or certain novel biofilm inhibitors that could provide a breakthrough specifically targeting the biofilm associated fungi.

2nd International Conference on Infectious Diseases and Nanomedicine – 2015 (ICIDN-2015)
December 15-18, 2015, Kathmandu, Nepal

Invited Lecture (IL) -11

Demonstration of the Unbearable Lightness of Phage Therapy Targeting Resistant Bacteria

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Multi-drug resistant (MDR) microorganisms continuously challenge antibiotics' efficacy. Consequently the development of non-antibiotic based alternative treatment is paramount. A promising approach is bacteriophage (phage) therapy, a process in which phages are employed to treat bacterial infections. Due to the ease with which they are isolated, their ability to destroy biofilms, their high specificity and dynamic reproduction in correlation with their target, phage therapy that was abandoned is now revisited. Previously we isolated bacteriophages, termed EFDG1, which had successfully eradicated a vancomycin resistant strain of *Enterococcus faecalis* (V583), a life threatening pathogen found in many infections. Moreover, this pathogen was defined by the CDC as a major threat as it easily developing resistance to the last resort antibiotics. While working with EFDG1, a resistant mutant (*EF_EFDG1)* emerged. With antibiotics, this situation is almost hopeless. However, with phage therapy, we quickly isolated another phage (EFLK1) that efficiently killed *EF_EFDG1*. Both phages were visualized by electron microscopy and their coding sequences were determined by whole genome sequencing revealing that they both belong to the Myoviridae family of phages. The antibacterial efficacy of the phages was evaluated *in vitro* against planktonic and biofilm cultures. Interestingly, despite their similarity their antibacterial activity was different. While EFDG1 efficiently eradicated *E. faecalis* in logarithmic phase, EFLK1 highly affected bacteria in stationary phase. In conclusion, this work show that in contrast to antibiotics treatment, the evolvement of resistant bacteria is not that problematic. Therefore, we believe phage therapy using cocktails of phages is a promising approach to treat hard to eradicate infections.

Invited Lecture (IL)-12

Intelligent Polymeric Micro/nanoparticles with Entrapped Active Agents

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Natural polymer-based micro/nanoparticles have been exploited as carriers for drug delivery. Spherical and needle like carrier particles with varied sizes (nano/micro) and shapes (spherical/needle like) and morphology (porous/nonporous) were synthesized by using natural polymers from renewable resource. Micro/nanoparticles with encapsulated solid and liquid active agents were prepared by using polyelectrolyte complexation of natural polymers gelatin and sodium carboxymethyl cellulose/sodium alginate/carrageenan. %yield, viscosity and turbidity measurements were carried out to evaluate the pH and ratio of the two polymers that produced highest yield. The encapsulation efficiency of oil and drug were dependent on the amount of crosslinker, oil/drug loading and polymer concentration. Scanning electron micrographs showed the formation of free flowing spherical micro/nanoparticles in case of isoniazid loading and a bit agglomerated in case of oil loading. The particles were found to be pH responsive. The size of the micro/nanoparticles tends to increase with the increase of the concentration of the polymer. Thermogravimetric analysis showed the improvement of thermal stability with crosslinking. Fourier Transform Infrared Spectroscopy study showed that there was no significant interaction between oil/drug and polymer complex.

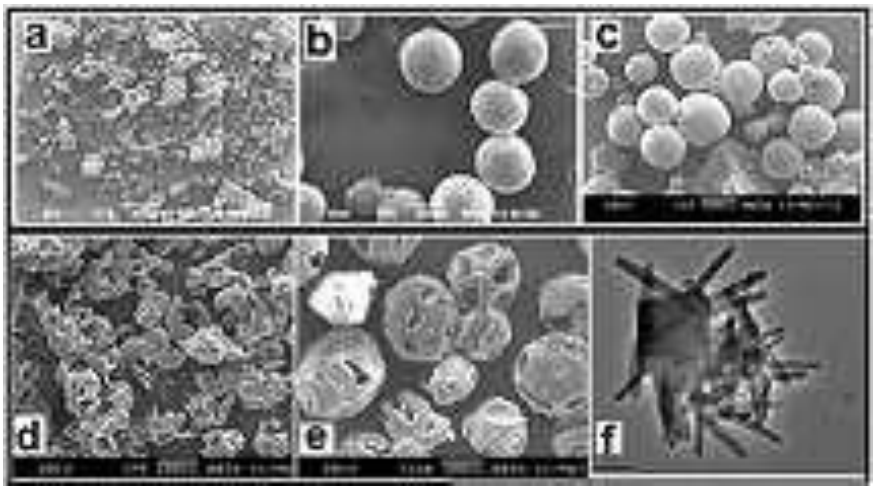

Figure 1. Representative SEM and TEM images of the polymer micro/nano particles

The drug-carrier particles were further characterized by TEM, DLS, NMR, XRD and DSC studies. The natural polymeric micro/nano carriers showed immense potential for drug delivery applications.

Oral Presentations

Symposium: Cellular and Molecular Microbiology of Infectious Diseases

Symposium: Epidemiology and Infectious Diseases Surveillance

Symposium: Antimicrobials, Vaccines and Alternatives

Symposium: Nanomaterials and Bio-medical Materials

Symposium: Drug Design, Drug Delivery and Tissue Engineering

Molecular Studies on the Haitian variant *ctxB* gene and cholera toxin production in *Vibrio cholerae* O1outbreak strains isolated from India

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Cholera, caused by a Gram negative bacterium *Vibrio cholera*e has been endemic in south Asia, especially the Ganges delta region in India from the time of recorded history. From previous studies, a shift in genetic and phenotypic level of the pandemic clones of *V. cholerae* was reported from all over the world. In this context, we planned to characterize the *V. cholerae* epidemic strains circulating in the country. In the present investigation, a set of *V. cholerae* outbreak strains from different geographical locations of south India is assessed to understand its molecular nature. Virulence gene profiling and antibiotic resistance pattern of these strains were analysed. Major virulence gene *ctxB* was sequenced. Amount of cholera toxin produced was assessed by GM_1ELISA and their ability to persist in the environment was studied by employing biofilm assay.

In our study, *ctxB* gene revealed an additional mutation at 58^{th} position similar to that of the strains isolated from Haiti in 2010 and possessed all major virulence genes. GM_1 ELISA revealed that these variant strains produced cholera toxin in the range 18000-78000 ng/ml with majority of strains producing cholera toxin lesser than Classical (40000-80000 ng/ml) but higher than the prototype El Tor strains (9000-40000 ng/ml).The difference in cholera toxin production was statistically analysed by two- tailed *t* test, the P value being <0.05 with reference strains. Biofilm assay revealed majority of strains assessed are poor biofilm formers but multidrug resistant against Trimethoprim, Co- trimoxazole, Streptomycin, Nalidixic Acid, Ampicillin and Erythromycin. Our results revealed that *ctxB* variant strains similar to that of Haitian outbreak isolates were circulating in India even before the outbreak in Haiti. We assume that the understanding of pathogenicity and antibiotic resistance pattern of the new variant strain can help us to be better equipped to treat/control cholera in a more efficient way.

Characterization of *Staphylococcus aureus* strains from skin and wound infection cases in Haripur and Abbotabad cities of Pakistan

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Staphylococcus aureus is a bacterial species responsible for a high number skin and wound infections. Treatment of these infections has been complicated by emergence of antibiotic resistant strains. *S. aureus* strains tend to be resistant against a number of prescribed antibiotics such as methicillin, doxycycline, ciprofloxacin, ofloxacin, erythromycin, vancomycin, ceftriaxone, lincomycin and augmentin. Recent studies from different countries report that majority of the strains of this bacterial species have acquired resistance against penicillin class of antibiotics including penicillinase resistant penicillin called methicillin. In the present study 102 *S. aureus* strains were isolated from skin infection cases. Antibiotic sensitivity testing was carried out by using disc diffusion assay. Presence of Panton Valentine Leukocidin (PVL) and *Staphylococcus* Cassette Chromosome (*SSCmec*) genes were determined by using PCR. The results of our study show high level of antibiotic resistance in the isolated strains. Over 70% are resistant to methicillin and about 15% of them bear genes encoding for PVL. Injudicious use of antibiotics may be responsible for dissemination of antibiotic resistance. Antibiotic resistance profiles of the isolated strains are also presented.

Prevalence of Human Papilloma Virus among Women Visiting a Health Facility in Kathmandu, Nepal

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Infection with Human papillomavirus (HPV) is one of the sexually transmitted infections that constitute a global public health concern. With the objectives to determine the prevalence of HPV infection and its associated factors, a prospective study was done from February 2014 to January 2015. A total of 101 cervical scrapes of women ages 25-65 years were collected and processed at Annapurna Neurological Institute and Allied Science, Kathmandu for investigation. Ethical review committee of Shigan Health foundation approved the study. Polymerase chain reaction (PCR) was used to amplify a region in HPV L1 gene using GP5+/6+ primers, and specific primers for specific genotype detection. Statistical analysis was performed with Statistical Packages for Social Science (SPSS) version 20. Over all prevalence of HPV infection was 32.7% (33/101). Among HPV positive, HPV16 was most prevalent (66.7%) followed by HPV18 (30.3%), HPV33 (3.0%) and HPV58 (3.0%). The median age of HPV positive women was 42 years. The peak HPV prevalence of 54.2% (13/24) was in women aged 41-45 years. The prevalence of HPV infection among women having >2 parity was found higher (47.2%; 25/53) compared with women with ≤2 parity (16.7%; 8/48) ($P<0.05$). The HPV prevalence was significantly higher in women who were smokers, illiterate and married at or below 15 years of age ($p<0.05$). The HPV infection was found high among women with hypertrophied cervix, tinges blood discharge, pelvic-pain and irregular menstruation ($p<0.05$). There was no significant association between use of contraceptive, co-infection and HPV infection in the study population. These findings highlight the necessity of regular monitoring, surveillance of HPV infection and development of appropriate public health programmes.

Community and hospital acquired Methicillin resistant *Staphylococcus aureus* (MRSA): association of Panton Valentine Leukocidin (PVL) genes

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Methicillin resistant *Staphylococcus aureus* (MRSA) is a major human pathogen associated with nosocomial and community infections. Panton Valentine Leukocidin (*PVL*) is considered as one of the important virulence factors of *S. aureus* responsible for destruction of white blood cells, necrosis and apoptosis and generally used as marker for community associated MRSA (CA-MRSA). This study was aimed to determine the prevalence of *PVL* genes among MRSA isolates and to check the reliability of *PVL* as marker of community acquired MRSA. A total of 139 MRSA strains were isolated from clinical specimens and various hospital units (Operation Theater and Intensive Care Units) of Manipal Teaching Hospital, Pokhara, Nepal. Antibiotic susceptibility testing was performed by disc diffusion method according to CLSI guidelines. Multiplex PCR was used to detect *mecA* and *PVL* genes. Out of 139 MRSA isolates, 83 (59.7%) were community acquired and 56 (40.3%) were hospital acquired. Out of 139 MRSA isolates, 79 (56.8 %) were *PVL* positive. Among the CA-MRSA (n=83), the majority (n=75) were *PVL* positive, while *PVL* was detected only in 4 (7.1%) hospital associated MRSA according to the clinical criteria used. None of the MRSA isolates from hospital environment showed *PVL* genes. The majority of the *PVL* positive strains (n=74) 75.5% were isolated from pus samples. Antibiotic resistance among *PVL* negative MRSA isolates was found higher as compared to *PVL* positive MRSA but was not statistically significant except in case of erythromycin (p value=0.021). Our study showed high prevalence of *PVL* among CA- MRSA isolates. This is the first study from Nepal, testing *PVL* among MRSA isolates from hospital origin. The absence of *PVL* among MRSA isolates from hospital environment indicates its poor association with hospital acquired MRSA and may be used a marker for community acquired MRSA in Nepal.

Heart and liver regeneration in zebra fish using silver synthesis particle from Marine Plant- *in vivo*

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The zebrafish, *Danio rerio*, is a small teleost fish originating from the rivers of northern and eastern India (Engeszer et al. 2007). It possesses a number of advantageous physical characteristics that have resulted in its common use today as a laboratory model. The present study was aimed to identify the heart and liver regeneration in zebra fish using biosynthesis silver nanoparticles from *Sargassum sps.* Of the selected seaweed extract showed the maximum synthesis of silver nanoparticles. This work focused on the activity of these compounds when incorporated into the zebrafish (*Danio rerio*) system. We began investigating the *in vivo* assay effect of these Hepatocyte Viability Staining After H_2O_2 Treatment, Cardiomyocyte Response to Ca^{++}, Cardio vascular heart rate activity by measuring hypertrophy, Cardio vascular pathology and cardio vascular regeneration, Liver regeneration and Liver pathology, Molecular pathway target identification and Hypothesis on Interacting Domain ($Agno_3$) of the vertebrate model organism. The FTIR results of most potent leaf extract-synthesized silver nanoparticles showed the prominent peaks (range between 620.967 to 2,854.14) Further, the results of XRD analysis showed the 2h intense values (38.11 and 70.57) within the ranges of Bragg's reflection. In addition, the SEM analysis showed the results of particle sizes (50–100 nm). It can be concluded from the present findings that, the biosynthesis of silver nanoparticles from the seaweed extract of *Sargassum sps.* Can be used as potential exploring its cardioprotective and liver protective ability using zebra fish as model organism.

Synthesis, Spectroscopic Characterization and Insulin Mimetic Activity of Oxovanadium(IV) Macrocyclic Complexes

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Oxovanadium(IV) complexes of triazoles and their substituted derivatives have biological and medicinal properties. In search of designing metal based biologically active agents that could work against diabetes, biological and medicinal properties of triazoles/substituted triazoles and vanadium metal found vital to join the chemistry of both moieties. A new series of oxovanadium(IV) macrocyclic complexes have been synthesized by the reactions of Schiff bases, derived from 3-(phenyl/substituted phenyl)-4-amino-5-hydrazino-1,2,4-triazole with salicyldehyde/*o*-hydroxyacetophenone and 1,4-dibromobutane in the presence of oxovanadium(IV) sulphate solution in ethanol. Complexes were well characterized on the basis of analytical data, magnetic susceptibility, UV-Vis and IR data. The X-band EPR spectra of all the complexes have been recorded in room and liquid nitrogen temperature. Powder X-ray diffraction pattern shows the size of the particles to be 31-90 nm range. Insulin mimetic behavior of $[C_{26}H_{24}N_6O_3V]SO_4$ macrocyclic complex has been studied in streptozocin induced diabetic rats over a period of 28 days. In order to study the insulin mimetic behavior body weight, blood glucose level, cholesterol and plasma creatinine are taken into consideration. The administration of the complex in diabetic rats showed the insulin mimetic behavior.

ZnO Nanoparticles and its Medical Applications in Cancerous Cell

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The addition of nanotechnology with biology provides the opportunity for the development of new materials at the nanometer size range that can be used for many potential applications in biological science and clinical medicine [1]. During the reduction at nanoscale area, unique size-dependent properties of nanomaterials, including nanoparticles (NP), are manifested. M a t e r i a l s that utilize these characteristics are very attractive for use in novel biomedical applications.

In our present study, we have observed the effect of ZnO nanoparticles in biological system. Here, we synthesize such kind of ZnO nanoparticles which shows the strong response to cancerous cells. The entry of the nanoparticles into the tumor tissue and their subsequent retention can be easily facilitate depends on the size, by the process named as the enhanced permeation and retention (EPR) effect. One of the primary advantages for considering ZnO nanoparticles for use in cancer is the inherent preferential cytotoxicity against cancer cells in vitro. It is anticipated that their cancer cell selectivity may be even further improved by engineering design to minimize harmful effects to normal body cells, which has been observed to occur at very high concentrations of ZnO nanoparticles, specifically in the smaller size range of 4–20 nm. Rapid thermal chemical vapor deposition (RTCVD) [2] was used to prepare various nanostructures of ZnO.

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Nanomaterials based electrochemical biosensors for medical applications

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Electrochemical biosensors have attracted considerable attention for the sensitive detection of variety of compounds i.e. disease biomarkers, antigens, toxins and drugs. These devices are uniquely qualified for meeting the small size, cost effectiveness, and power requirements of decentralized testing and show great promise for a wide range of biomedical and environmental applications. The attractive properties of nanomaterials, due to high surface area to volume ratio, have provided the platform for the fabrication of wide range of electrochemical biosensors that exhibit improved analytical capacities. Carbon based nanomaterials, including carbon nanotubes, C_{60} and graphene, and gold nanoparticles, have garnered tremendous interest for their potential in the design of high-performance electrochemical biosensor platforms due to their exceptional thermal, mechanical, electronic, and catalytic properties. A new approach for the electrochemical detection of some key disease biomarkers: glucose, cholesterol, thyroglobulin, and some pharmaceutical compounds: acetaminophen and valacyclovir have been investigated based on the co-immobilization of horseradish peroxidise, glucose oxidase, cholesterol oxidase (ChOx), cholesterol esterase (ChE), and methylene blue on the functionalized single-walled carbon nanotubes (SWCNTs), reduced graphene oxide (rGO), SWCNTs-rGO, nanoporous gold nanoparticles, and buckypaper as a novel sensing materials. The resulting biosensors showed excellent electrocatalytic activity to these biomarkers at physiological pH 7.4. The practical applications of the developed biosensors have been studied by the determination of these compounds in biological samples spiked in human plasma AB. The sensitive, reliable and rapid analyses of critical disease biomarkers and globally emerging pharmaceutical compounds at nanomaterials based electrochemical biosensor platforms may enable an extensive range of applications in preemptive medical diagnostics.

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Trick malaria parasites using nanomimics of host red blood cell membranes?

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The threat of emerging and spreading drug resistance as well as limited availability, efficacy or a total lack of vaccines highlights the need for innovative strategies to combat infectious diseases. Malaria is such an example; the *Plasmodium* parasites, causing this disease, are responsible for more than 200 million infections – killing about 600'000 people each year – with *Plasmodium falciparum* being the most dangerous species of this pathogen.[1]

Our nanotechnological approach to tackle this disease aims for a drug- and vaccine-like dual action.[2,3] We designed simple polymer-based nanostructures that mimic host red blood cell (RBC) membranes (nanomimics) by exposing a known receptor, important for the initial attachment of *Plasmodium* merozoites to RBCs, on the surface of the nanostructure. We used different light-, fluorescence- and electron beam-based methods to characterize these nanomimics as well as to study the interaction of nanomimics with *Plasmodium* proteins and whole malaria parasites. Typical characteristics of the nanomimics are a membranous, vesicular structure with a membrane thickness of about 10 nm and diameters of 130 ± 30 nm. Furthermore, nanomimics successfully interacted with the parasite protein *Pf*MSP1$_{42}$, which is the parasite ligand that is known to interact with heparan sulfate on RBCs.[4] When added to a malaria parasite culture, nanomimics very efficiently interrupted the life cycle of the parasites by inhibiting RBC invasion (drug action, Figure 1). In fact, one nanomimic was about 10'000 fold more potent when compared to a soluble receptor molecule.[2] If successful *in vivo*, this strategy will expose a huge number of merozoites to the immune system, which could significantly boost the immune reaction against the free parasites (vaccine-like action).

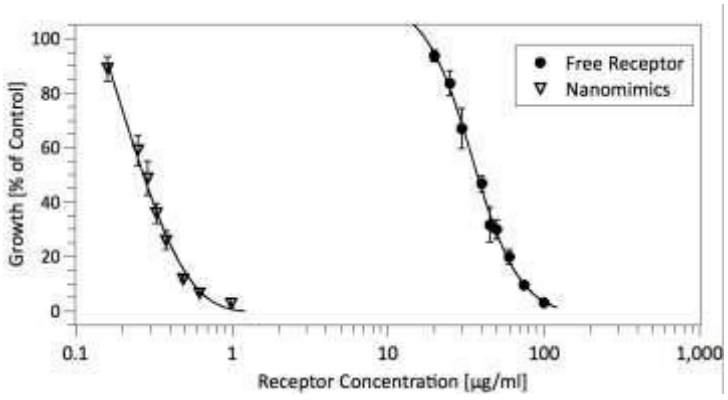

Figure 1. *In vitro* efficacy of nanomimics and soluble receptors against malaria presented as dose-response curves. (Modified with permission from ACS).[2]

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Antibacterial and cytotoxic evaluation of different extracts of *Parthenium hysterophorus*

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Parthenium hysterophorus is a fast growing weed frequently spotted on abandoned lands, road sides in Pakistan. This weed has not yet been revealed economically in Pakistan. The present study was designed to determine the antibacterial activity of aqueous, Methanolic, ethanolic, and chloroformic extracts of *Parthenium hysterophorus.* Further cytotoxic effects of extracts having maximum antibacterial activity were also determined to get an idea regarding the therapeutic index of extracts and to determine its safety. For this purpose extracts were prepared using water, methanol, ethanol and chloroform. Antibacterial sensitivity of different concentrations (1, 10, 25, 50 and 100mg/ml) of all extracts was evaluated by well diffusion method against *Staphylococcus aureus,* MRSA (Methicillin resistant *Staphylococcus aureus), Bacillus subtilis, Escherichia coli, Pseudomonas aeroginosa* and

Klebsiella pneumonia. Minimum Inhibitory Concentration was determined by broth dilution susceptibility assay. The extract having maximum antibacterial activity was then evaluated by using *In vitro* MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay on Vero cell lines in 96 well plate having M-199 cell culture media and different concentrations (3.125, 6.25, 12.5, 25, 50,100, 200, 400, 800,1600µg/ml) of all extracts. Culturing media was used as positive control while 20% DMSO was used as negative control. The cells along with different concentrations were incubated for 48hrs at 37°C. Viability of cells was determined by calculating the cell survival percentage.

Antibacterial activity of ethanolic extract was maximum in comparison with all other three extracts against chosen bacteria. MICs of Gram positive bacteria were in range 100-200 µg/ml and 50-100 µg/ml that of Gram negative bacteria. Cytotoxic evaluation revealed that concentrations of aqueous extract >6.25 µg/ml, ethanolic extract >50µg/ml, methanolic extract >50 µg/ml and chloroform extract >I 2.5µg/ml were cytotoxic to Vero cells. Thus, *Parthenium hysterophorus* do exhibit antibacterial activity but it might not be used as antibacterial agent due to its cytotoxic potential against normal cells *in vitro*.

Epitope -based peptide vaccine design and target site depiction against Ebola viruses

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Ebola viruses (EBOVs) have been identified as an emerging threat in recent year as it causes severe hemorrhagic fever in human. Epitope-based vaccine design for EBOVs remains a top priority because a mere progress has been made in this regard. Another reason is the lack of antiviral drug and licensed vaccine though there is a severe outbreak in Central Africa. In this study, we aimed to design an epitope-based vaccine that can trigger a significant immune response as well as to prognosticate inhibitor that can bind with potential drug target sites by using various immunoinformatics and docking simulation tools. The capacity to induce both humoral and cell-mediated immunity by T cell and B cell were checked for the selected protein. The peptide region spanning 9 amino acids from 42 -50 and the sequence TLASIGTAF were found as the most potential B and T cell epitopes respectively. This peptide could interact with 12 HLAs and showed high population coverage up to 80.99%. By using molecular docking, the epitope was further appraised for binding against HLA molecules to verify the binding cleft interaction. In addition with this, the allergenicity of the epitopes was also evaluated. In the post therapeutic strategy, docking study of predicted 3D structure identified suitable therapeutic inhibitor against targeted protein. However, this computational epitope-based peptide vaccine designing and target site prediction against EBOVs opens up a new horizon which may be the prospective way in Ebola viruses research; the results require validation by *in vitro* and *in vivo* experiments.

Prevalence of extended spectrum beta-lactamases (ESBLs) among uropathogenes at NU hospital Bangalore

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Urinary tract is the second most common site of bacterial infections among chronic kidney patients. Gram-negative bacteria (GNB) that possess extended spectrum β-lactamases (ESBLs) genes have proven to be a concern to the medical community because of their high resistance rates to 3rd generation cephalosporins. ESBLs production has been associated with higher morbidity and mortality rates and has been reported in *Escherichia coli* and *Klebsiella pneumoniae*. ESBLs are emerging worldwide, making rapid and adequate ESBLs detection crucial for the choice of correct antimicrobial therapy. To determine the profile of uropathogen, their antibiogram and detection of ESBLs producing strains. Isolation, identification of organism was done by standard microbiological procedure as described in CLSI. Antibiotic susceptibility & production of ESBL was detected by BD Phoneix^{TM} 100 (Becton&Dickinson New Jersey, USA). In this hospital based cross-sectional study 5066 midstream urine samples were collected from April 2014 to April 2015 from clinically suspected UTI patients. Significant bacteriuria was present in 1687 [33%]of specimen. The most common pathogens isolated were *Escherichia coli* 903 *(*53.52%). It was found 946 (56.12%) of Gram-negative uropathogens were ESBLs. Majority of ESBLs seen in *Escherichia* (92.80%) The ESBLs producing *Escherichia coli* were highly susceptible to imipenem (90.90%) and meropenem (94.45%). *Escherichia coli* is the commonest cause of UTI. Majority of UTI are mono-microbial. Screening of multidrug resistant bacteria especially GNB poses considerable therapeutic challenges in critical care patients because of the production of ESBLs.. Antibiotic stewardship programme and active surveillance of hospital circulating strains is need of hour to combat this emergent situation.

Salmonella enterica serovar Typhi in Carcinoma Thyriod: A Case report

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S. enterica serovar Typhi an intracellular gram negative enteric bacilli seeds and lodges to form localized infection and abscesses in various sites in the body. We report the isolation of *S.enterica* serovar Typhi from the thyroid abscess of a female patient diagnosed with papillary carcinoma (follicular variant) of thyroid.

A 30 year old female patient came with history of increased swelling in the thyroid region since a year associated with increased night temperatures and headache and difficulty in swallowing. Pus from drain tube after third post-operative day from the thyroid region was sent for microbiological work up. Pure culture of non-lactose fermenting colonies on MacConkey agar and mucoid grey colonies on blood agar were seen after overnight incubation at 37º. Cultures were identified as *S.enterica* serovar Typhi by manual biotyping (IMVIC reaction) and confirmed by slide agglutination with "O" "H" and factor 9 anti-sera and reconfirmed. The isolate was sensitive to routine antibiotics including co-trimoxazole and ciprofloxacin. Pathological investigations of thyroid tissue for frozen section and fine needle aspiration cytology was papillary carcinoma (follicular variant). *S.enterica* serovar Typhi presenting as localized abscess such as in hepatic, spleen, bones, intracranial, skin, breast have been reported in non-cancerous patients but *S. enterica* serovar Typhi a sensitive strain presenting as localised abscess in thyroid in a thyroid carcinoma patient is the first report in literature.

Malignancy could be one of the cardinal risk factor for extra intestinal manifestation of enteric pathogens. To conclude *S.enterica* serovar Typhi in this patient may be implicated as a carrier vehicle for carcinogenesis.

Candida parapsilosis onychomycosis, an unusual presentation in a child

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Onychomycosis is seen in approximately 20% of people in East Asia. Though dermatophytes are important causative agents, *Candida parapsilosis* is emerging as a potential pathogen. Patients present with thickened, brittle nail, ridging, discoloration of both nail and nail bed. Nail becomes friable, may disintegrate, and if untreated, may be lost. A 4 year old male child was admitted with features of cystitis and was treated with broad spectrum antibitoics, such as cefixime and gentamicin parenterally for 10 days. He recovered within a week. However, he developed yellowish discoloration with clipping of nails of both hands within a span of seven days following discontinuation of antibiotics. The sloughed out nail, on microscopy, showed numerous yeast cells that were identified as *Candida parapsilosis* by the conventional techniques and later, confirmed by DNA sequencing. Yeast cells with coarse pseudomycelia (giant forms) were demonstrated under hematoxylin and eosin(H&E) stain as well. MICs (ug/ml) of fluconazole (8), voriconazole (0.1), anidulafungin (0.3), caspofungin (0.1), micafungin (4), amphotericin B (2), itraconazole (0.3) and posaconazole (0.1) for the organism were estimated according to CLSI guidelines. Patient was successfully treated with topical fungizone and oral fluconazole, with marked improvement in nail discoloration, with formation of granulation tissue, and restoration of vascularity in the nail bed. Onychomycosis is not an infrequent clinical problem at the extremes of ages, irrespective of the immune status of the patient. Use of broad spectrum antibiotics may or may not have much implication in the predisposition of such infections. Hence there should always be a high index of suspicion of *Candida parapsilosis* onychomycosis, especially when a patient in the pediatric age group, presents with unusual features.

Microsporidial keratitis in immunocompetent patients from North India

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Keratitis is a second leading cause of blindness in both developing and developed countries after cataract. Various microorganisms such as bacteria, fungi, viruses and parasites can cause keratitis. Parasitic corneal pathogens viz. *Onchocerca, Leishmania, Trypanosoma, Acanthamoeba*, have been well described with recent attention focused on microsporidial keratitis. At present, microsporidial keratitis is under-diagnosed and under-reported and early diagnosis is essential for better management. A total of 260 patients with suspected infectious keratitis presenting to the Advanced Eye Centre at Postgraduate Institute of Medical Education and Research, a tertiary care centre of North India were included. Their corneal scrapings and tear samples were collected for microscopic examination and molecular diagnosis. A total of 2 patients (0.76%) were diagnosed with microsporidial keratitis by Calcofluor white and Modified trichrome staining and one sample (0.38%) by PCR. Both the patients had history of mud/soil exposure and trauma with vegetative matter. Both cases presented with recurrent keratitis of seven to eight months duration with non-healing epithelial defect and deep stromal infiltrate. One patient underwent corneal transplant while other did not turn up for follow up. Thus, microsporidial keratitis should be considered in differential diagnosis of keratitis especially following trauma to eye.

Conventional Methods Of Methicillin Resistant Staphylococcus Aureus (MRSA) Detection

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The global burden of Staphylococcus aureus infections is increasing with the emergence of resistance towards methicillin and other drugs of routine therapeutic use. The study was performed with an objective to detect methicillin resistance in S. aureus isolated using the conventional cefoxitin disc diffusion and oxacillin agar dilution method. A total of 661 specimens were processed in Dhulikhel hospital from July 2013 to January 2014 following the standard microbiological protocol. The isolates were tested for the antibiotic susceptibility and methicillin resistance by modified Kirby-Bauer Disc Diffusion method. The MIC value of oxacillin was determined by agar dilution. Of the total growth positive cultures (489, 73.97%), 62.98% showed S. aureus. A total of 17.90% isolates were methicillin resistant by cefoxitin disc diffusion assay. The most effective drugs were chloramphenicol and cloxacillin for MRSA (100%) and MSSA (99.60%) respectively. The agar dilution showed only 14.90% MRSA with the MIC50 values 0.25µg/ml and 8µg/ml for the MSSA and MRSA respectively. Phenotypically, oxacillin agar dilution is found to be reliable than cefoxitin disc diffusion method for the detection of methicillin resistance; however, result suggests the molecular detection of mecA gene for the accurate detection.

Evaluation of Multiplex PCR using *MPB64* and *IS6110* primers for Rapid Diagnosis of Tuberculous Meningitis

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Tuberculous meningitis is one of the most serious clinical conditions of extra pulmonary tuberculosis. Proper diagnosis and treatment methods are needed for its better management. It is difficult to diagnose due to a lack of rapid, sensitive, and specific tests. Newer methods, which are easy and reliable, are required to diagnose TBM at an early stage. Thus our aim was to evaluate the Multiplex polymerase chain reaction (PCR) technique, using primers directed against the insertion sequence *IS6110* and *MPB64* gene, for the detection of Mycobacterium tuberculosis in the CSF, for the diagnosis of TBM patients. 102 CSF samples were analyzed from patients with 'suspected' TBM and a control group of 10 patients including other neurological disorders. CSF sediments were analyzed individually for *M. tuberculosis* DNA by Multiplex PCR using two set of primers targeting insertion sequence *IS6110* and gene MBp64, which is very specific for MTBC. Thus generated data were analyzed using statistical software SPSS version 20 and Kappa test were applied. Out of 37 patients diagnosed TBM clinically *MPB64* PCR was positive in 22, *IS6110* PCR positive in 28, Both PCR using Multiplex positive in 34 and Microscopy in 1. Thus positivity of *MPB64* PCR, *IS6110* PCR, Multiplex PCR using both *IS6110* and *MPB64* primers and Microscopy were 62.3%, 75.4%, 91.8% and 2.7% respectively. In non TBM group PCR was negative in all cases hence, the specificity was 100%. Use of multiplex PCR system, using primers targeting *IS6110* and *MPB64*, for the detection of *M. tuberculosis* DNA in CSF samples, has high positivity than any one of them alone, and could be used for the early detection of TBM in CSF samples.

A Molecular-Beacon-Based asymmetric PCR assay for easy visualization of amplicons in the diagnosis of trichomoniasis using pyruvate:ferredoxinoxidoreductase proprotein (pfoB) gene as target

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Conventional polymerase chain reaction (PCR) based diagnostics assays are accurate, quick and confirmative for detection of microorganism in clinical samples with superior sensitivity than traditional culture-based microbiology assays. Unfortunately, these advantages are partially dissipated due to technical factors and are accessible only to selected population. The assays are also associated with some degree of false positive and carry risk of cross contamination by gel electrophoresis, which is cumbersome and protracted. Hence, a PCR assay with easy visualization of the amplified product will be profitable. We developed a rapid, sensitive, specific PCR based assay where in the amplicons could be visualized effortlessly. Dry ectocervical swabs (n=392) from symptomatic female patients with vaginal discharge visiting Department of Obstetrics & Gynecology of Safdarjung Hospital, New Delhi were collected and an in-house PCR assay using primers against *pfoB* gene of *T. vaginalis* was developed. The performance and reproducibility of PCR assay was evaluated by composite reference standard (CRS) method using previously established PCR primers, *18S rRNA*, β-*tublin*, *pROS21*. For easy visualization of the amplified product, molecular beacon specific to the internal region of the amplicon was designed.

PCR product could be directly visualized using fluorescent dark reader or by MicroPlate Reader. The beacon based assay is highly specific as confirmed by competition experiments and highly sensitive as can detect as low as 20fg of genomic DNA (3–4 pathogens). The minimum infrastructure requirement and ease to perform the assay makes this PCR method highly useful for resource poor countries for better disease management.

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Next generation sequencing to divulge genomic framework of *Helicobacter pylori*

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H. pylori is a gram negative spiral bacilli, infecting 50% of the world population. It is related to variety of upper gastrointestinal disorders, such as chronic gastritis, peptic ulcer disease, gastric mucosa associated lymphoid tissue (MALT) lymphoma, and it has been classified as the class 1 carcinogen for gastric cancer by World Health Organization. *H. pylori* exhibit a high level of genetic variation due to a number of factors like mutation, intraspecific genetic recombination and its long evolutionary history. Here we present a model of using Illumina shotgun sequencing platform to unravel the genome of *H. pylori.* In this study we have sequenced genome of 100 clinical *H. pylori* isolates with different host ethnic backgrounds and analysed the population genetic variance. We have also studied the prevalence of the whole cagPAI and its genotype across the different *H. pylori* population as well as other virulence genes, such as *vacA, babAB, iceA, dupA,* and *ureAB*. In addition, we have reported the SNPs that are associated with the antibiotic resistance. These genomic information from Next Generation Sequencing open up new areas for the better understanding of the mechanism of pathogenesis of *H. pylori*. They may be used to develop personalized regimen for *H. pylori* to improve current treatment efficacy varied by individual unique bacterial genetics background.

Gene expression analysis of *Plasmodium falciparum* Dd2 strain using Whole Transcriptome Sequencing

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Drug resistance in *Plasmodium falciparum* is a global health burden as it reverses the malaria control achieved so far. Thus, understanding the gene expression profile will reveal us the mechanism of drug resistance involved, thereby aiding to develop potent drug/vaccine targets for malaria treatment and detection of drug resistance. In our study, we have used the whole transcriptome sequencing to typify the gene expression profile of 48 h intraerythrocytic stage of chloroquine resistant *P. falciparum* strain (Dd2). The strain was cultured by *in vitro* method and harvested at 48 h intraerythrocytic stage having 5% parasitemia. Total RNA extracted from the culture using TRIzol method, and whole transcriptome sequencing was performed using Illumina HiSeq 2500 platform with paired-end reads. The reads having Phred quality score more than 30 were used for further analysis. The RNA contaminations (i.e. rRNA, ncRNAs etc.) were also removed from the data. The reads were then aligned with the reference *P. falciparum* genome from Ensemble Genome database (Release 26) using Tophat program (version 2.0.8) and the alignment percentage was 89.13% for Dd2 strain. After aligning the reads with reference gene model, the aligned reads were used for estimating expression of the genes and transcripts using cufflinks program (version 2.0.2). More than 60% of the genes have unknown function and uncharacterized as per the gtf file information that is available for the species. Approximately 5% of the genes act as transporter and involved in transmembrane transport of metabolite, drugs, etc. Also, ~5% of genes are involved in protein translation mechanism and ~4% of genes are associated with RNA metabolism/ transcription process. Moreover, ~3% of genes are surface antigens (RIF, VAR), involved in antigenic variation, pathogenesis and host-parasite interaction. Thus, whole transcriptome analysis explores the expression of genes in *P. falciparum* Dd2 strain compared to reference genome.

Detection of rpoB gene mutation in *Mycobacterium tuberculosis* by Amplification Refractory Mutation System-Polymerase Chain Reaction

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Multidrug-resistant tuberculosis (MDR-TB) is becoming a serious worldwide threat including Nepal. MDR-TB refers to the diseased condition whereby *Mycobacterium tuberculosis* becomes resistant to the first line of drug treatment i.e. rifampin and isoniazid. Resistance to rifampin (RIF) is mainly caused by the mutations in the *rpoB* gene which codes for the β-subunit of RNA polymerase. The amplification refractory mutation system (ARMS) PCR technique was used to detect mutations in the *rpoB* gene of *Mycobacterium tuberculosis* strains. Overall, DNA samples from 41 phenotypic MDR-TB were subjected to ARMS PCR using three different codon specific primers (516,526 and 531). These three codons occupy large portion of total mutation responsible for rifampin resistance. Out of the total DNA samples, 33 were bearing mutation in any of the three codons mentioned. In our study, the highest number of samples had mutation in codon 531 (96.97%) followed by codon 516(18.18%) and codon 526 (12.12%) respectively. Thus ARMS PCR can be used as an alternative diagnostic technique for detection of rifampin resistance in *Mycobacterium tuberculosis* strains in developing country like Nepal.

Identification and screening of novel antiretrovirals targeting HIV maturation

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Identification of new antiviral targets continues to be a high priority for development of HIV therapeutics. Maturation inhibitors represent an underdeveloped class of antiretroviral agents that block virus maturation by binding to the target of protease - Gag precursor ($Pr55^{Gag}$) and blocking a specific step in Gag processing. The first-in-class maturation inhibitor Bevirimat (BVM) binds to a pocket near CA-SP1 cleavage site in $Pr55^{Gag}$ and blocks CA-SP1 processing. Though BVM is a potent antiretroviral drug, naturally occurring polymorphisms within the vicinity of CA-SP1 cleavage site were responsible for resistance against it. We tested the efficacy of BVM and a structurally distinct compound PF-46396 for blocking the CA-SP1 processing in HIV-1 subtype C. We also tested efficacy of 5 new second-generation Bevirimat analogs (compounds GTP02-68 & 70, GTP03-16, 21 & 22) which contain modifications at C28 heteroatom. HIV-1 subtype B was included for comparison. Resistance mutations against compounds were selected *in vitro* by multi-cycle replication assay using human T cells HutR5 transfected with wild type molecular clone of HIV-1 subtype C K3016 in the presence of increasing concentrations of compounds. All experiments were performed in three replicates and comparisons were done using Student's t-test (CI 95%). Compounds were non-toxic within the concentrations (2 nM-5 µM) used in our experiments. BVM was found to be ineffective against HIV subtype C whereas PF- 46396 displayed dose dependent effect on inhibiting maturation. Bevirimat analogs were more potent against HIV subtype C strains than the parent compound BVM. Substitution mutations near CA-SP1 cleavage site rendering resistance against BVM analogs were mapped. This study identifies new BVM analogs that display higher efficacy relative to the parental compound BVM in their activities against both HIV 1 subtype C and B. These initial findings are likely to lead to the development of more potent and broadly active compounds.

High-resolution melt (HRM) analysis for rapid detection of *Mycobacterium leprae* drug resistance mutations from leprosy patients from India

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Currently, leprosy treatment and control is based on World Health Organization (WHO)-recommended multidrug therapy (MDT) and is in use for treatment of leprosy for the last 29 years. Using MDT the prevalence of leprosy has come down drastically all over the world. It has been noted from earlier experience that any therapeutic control measure for prevention of disease with antibiotics ultimately leads to emergence of drug resistance. Therefore, a surveillance mechanism should function as a 'watch dog' for identification of drug resistance.

For the detection of mutations within drug resistance-determining regions (DRDRs) of *folP1*, *rpoB*, and *gyrA*, targets for dapsone, rifampin, and fluoroquinolones, real-time Polymerase Chain Reaction (PCR)-HRM assays were done. Wild-type and drug-resistant mouse footpad-derived strains in a reference panel were tested. When tested in 50 sequence-characterized clinical strains, HRM identified all the mutants. Among these 50 samples 10, 6 and 3 samples were resistant to rifampicin, dapsone and ofloxacin respectively. Real-time PCR is a rapid technique (<3 h) including amplification and visualization. Having a high sensitivity as with conventional PCR, it has the added advantage of quantification to determine a cut-off that permits differentiation between mutant and wild type in the cases. Real-time PCR-HRM is a sensitive, simple, rapid, and high-throughput tool for routine screening of known DRDR mutants in cases at lower costs than current methods having the potential for quality control in leprosy investigations.

Studies on Degradable Polyester Based Composites for Biomedical Application

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Biodegradable polyester, poly (butylene adipate-*co*-terephathalate) (PBAT) is an interesting material and has been proved to be useful in numerous medicinal application. In this work, microcrystalline cellulose (MCC-JG) was extracted from agricultural waste wheat-stalk by a number of chemical treatments. PBAT was compounded with MCC-JG by internal melt mixing process. The PBAT/MCC-JG composites have MCC-JG in different weight ratios. These composites were characterized by tensile test, microindentation method, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and Fourier-transform infrared (FTIR) spectroscopy. The mechanical strength of the composites was found to be enhanced with MCC-JG upto 40 % loading whereas composites were found to be quite stable over a wide temperature range. The compatibility of the MCC-JG and PBAT was found to be good enough in the composites. Biodegradation of the composites was studied by soil composting. Microbial interaction with *Pseudomonas aeruginosa* showed typical color development in the composites showing the composites' potential for application as biosensor.

Synthesis of Magnetic Starch-Iron Oxide Nanocomposite for Controlled Drug Delivery

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In the field of medicine magnetic nanocomposite materials are gaining interest due to their excellent magnetic properties, stability, and good biocompatibility. In this study we have developed simple, *less* expensive, *less time consuming* method for the preparation of magnetic starch – iron oxide nanocomposite (abbreviated as SION). The magnetic SION were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), energy-dispersive X-ray analysis (EDX), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and vibrating sample magnetometer (VSM). An anti-tumor drug doxorubicin, was inserted in the composite through incubation mixing method. In addition, the drug release profile was studied at different pH, to determine the influence of the pH on the release of the drug. The drug release was monitored and quantified using the ultraviolet-visible spectrophotometer (UV-Vis), for doxorubicin. Results demonstrated that SION presented a good magnetic property, which enable the magnetic composite to target a specific place or tissue under the influence of external magnetic field. Furthermore, in the presence of acidic medium the release is comparably faster than in neutral pH which almost ceased at basic pH. At different pH the magnetic SION were able to perform a controlled pH stimulus release of drug. This phenomenon may be useful to perform a fine tuning of the system, allowing the easier adjust of the speed, site and amount of released drug, useful to improve medical treatments and even the welfare of the patients.

Biodegradability and antimicrobial properties of polyvinyl alcohol (PVA) based chitosan composites

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Chitosan, a natural biodegradable polymer, is of great interest in research field due to its excellent properties such as bioavailability, biocompatibility, non-toxicity and adsorption. The blends of chitosan and polyvinyl alcohol (PVA) have been reported to provide good mechanical properties, drug release control and an approach for producing polymeric packaging films for specific purposes. Therefore, the composites of polyvinyl alcohol with commercially available chitosan (CS-com) were prepared by solution casting method and characterized by Fourier transform spectroscopy (FTIR), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Characteristics absorption bands of polymer were observed in FTIR spectra of the blends. The biodegradability of PVA and its composites with chitosan were studied by vermi compost burial method. The biodegradability was tested by their physical appearance and weight loss method. Physical appearance and weight loss measurement showed that the composites have higher biodegradation rate in comparison to the pure PVA. The rate of weights loss of the pure commercial chitosan was found highest and least for pure PVA. The biodegradability was increased with the addition of chitosan. Further, the antimicrobial activity of PVA, chitosan and their composites were tested against the bacterias; *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* by inhibition zone method. The results showed that pure chitosan possess adequate antimicrobial activity whereas PVA/Com-cs composites were inactive toward.

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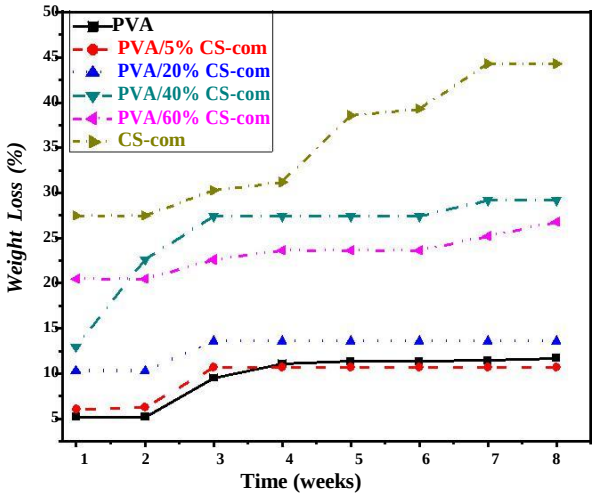

Figure 1. Plot of weight loss percentage *versus* time duration of PVA/chitosan composites

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Myxobacterial cytochromes P450 based whole cell biocatalyst: Generation of the novel drug derivatives from the tricyclic antidepressants, antipsychotics, antineoplastic agents and steroids

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Synthesis of regio- or stereoselective functionalizations of drug and drug-derivatives are one of the main goals of pharmaceutical research, which can be accomplished by radical reactions but are not selective enough to introduce the desired chiral alcohol function into those compounds. Since cytochromes P450 (P450s) are capable to insert oxygen into aromatic and aliphatic, activated and non-activated C-H bonds to generate pharmaceutically important molecules, there is a great scientific interest on microbial or eukaryotic P450s to employ as a biocatalyst. We have been engaged in studying the orphan P450s in the myxobacterium *Sorangium cellulosum* strain So ce56, since this genus is considered as the most promising resources for novel natural products which can synthesize antimicrobial macrolides and a novel class of antineoplastic agents like the anticancer drug epothilones. We have established P450 mediated *Escherichia coli/Bacillus megaterium* based whole-cell biocatalyst systems for the bioconversion of drug metabolites and the structure of the novel products were elucidated by 1D- and 2D-NMR spectroscopy using gs-HH-COSY, gs-HSQC, and gs-HMBC. CYP264A1, CYP267A1 and CYP267B2 were able to convert the tricyclic antidepressants and antipsychotics to known human drug metabolites in a milligram scale, revealing their ability to synthesize pharmaceutically important compounds for the first time. Likewise, CYP167A1, CYP265A1, CYP266A1 and CYP267B1 were able to convert the anticancer drug epothilone D to epothilone B, 14-OH-, 21-OH- and 26-OH-epothilone D, and 7-ketone epothilone D, respectively, in which the latter one represents a novel epothilone derivative and is a suitable candidate for pharmacological tests. In addition, for the first time, CYP260A1 was identified as 1α-hydroxylaze of C19-steroids, which provide an access to perform chemical modifications on the C-1 to generate isomers with C-1 and C-2 double bond because of the possible delocalization of the π-system during the release of a water molecule in the A-ring of the steroid, which is required for the synthesis of anabolic steroids and other steroidal drugs.

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Pakistani Black cobra snake venom: An Answer to various Infectious diseases

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Snake venom is a natural biological resource of several neurotoxic, cardiotoxic, cytotoxic, and other active compounds. Due to this broad range of biological functions, these biomolecules has been the subject of interest to the scientific communities. . Snake venom contains a variety of chemicals including pharmacological and toxicological properties. Microbial infections by bacteria, fungi, viruses and other parasites are among the 10 leading causes of death worldwide according to the World Health Organization (WHO). The presence and current emergence generate multiple resistant strains make the risk of these infections become more threatening as the treatment are now beyond reachable approach. In fact, resistance issues in pathogens has been the major factor responsible for increasing morbidity, mortality and health care costs of bacterial infections. The venom was obtained from Institute of Environmental and Pharmaceutical, DOW University of Health Sciences, Karachi Pakistan as a part of collaboration and then lyophilized. In this regard, in our research laboratory, antibacterial, antifungal and antiviral activities were performed. The results were quite promising against a number of bacterial and fungal pathogens. At the end of the present study, one can jump up the conclusion that snakes venom as few reports high lights possesses antibacterial, antiviral, antifungal potential that can be an ideal candidate that can fight against human critical pathogenic microbes. Moreover, some other exploration areas also will point out the role of snake venom as the therapeutic strategy against arthritis, inflammatory and pain disorders. Moreover, antioxidant activity was also good. Apart from the achievement of suggested results, further studies are surely required to figure out if there is a relationship between the venoms biocidal potentials and their enzymatic content. The active antimicrobial components should be purified, investigated for determination of their mechanism of action and examined over a wide range of microbial models. It is also recommended to sequence these antimicrobial proteins and use them as a prototype for drug design to present a new strategy for antimicrobial therapy depending on snake venoms and their derivatives.

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Loop-Mediated Isothermal Amplification (LAMP) For Rapid Diagnosis of Extrapulmonary Tuberculosis in 60 minutes

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Diagnosis of extra-pulmonary tuberculosis (EPTB) is highly challenging. Conventional techniques lack sensitivity and are time-consuming. Loop-mediated Isothermal Amplification (LAMP) is a promising nucleic-acid amplification assay. Rapid diagnosis of EPTB is required for early treatment and better patient outcomes. This reaction could be carried out in simple water bath under isothermal conditions in 60 minutes, eliminating the need for specialized equipment or expertise and can be performed in any laboratory and in rural settings in resource poor endemic settings. LAMP PCR targeting IS6110 specific region for *Mycobacterium tuberculosis* complex was evaluated for rapid diagnosis of EPTB. LAMP assay using six pairs of primers (IS6110) specific for *Mycobacterium tuberculosis* were performed on various EPTB samples of a total 110 patients (25 confirmed, 85 suspected) of EPTB and 50 non tuberculosis controls subjects. Microscopy, culture and IS6110 conventional PCR were also carried out these samples. LAMP PCR results were compared with IS6110 conventional PCR, culture and AFB smear results. LAMP test had sensitivity and specificity of 100% for confirmed EPTB cases. In 85 clinically suspected but unconfirmed EPTB cases, LAMP was positive in 72/85 (84.70%) cases. Sensitivity of IS6110 PCR in clinically confirmed and unconfirmed EPTB cases was 84% (21/25) and 72.94% (62/85) respectively. The overall sensitivity of microscopy, culture, IS6110 PCR and IS6110 LAMP test were 3.33%, 20%, 75.45%, and 88.18 % respectively. LAMP assay is promising technique for rapid diagnosis of EPTB in 60 minutes especially in resource poor endemic countries.

Risk evaluation of medical nanomaterials available in Mongolia

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Nano is a key technology to bring accelerated development in science and economy in 21st century. Besides lots of advantages contained in nanomaterials, cytotoxic effects may occur due to small size and large surface area. In last few years, human and environment exposure opportunity for this materials have been increased significantly due to uncontrolled consume and poor registration in the Customs of Mongolia. In this study, *in-vitro* toxicity of 21 samples including imported nanomedicines, disinfectant spray, cleaning solution and experimental products of Mongolia were determined. The cross correlation analysis and X-Ray diffraction analysis were used separately in order to determine particle size and its distribution. The mutagenicity was determined by Ames test for all samples. Heavy or other metals (As, Pb, Cr, Ni, Cu, Fe, Zn, Co, Se, Cd, Sb) as well as organic compounds were determined by AAS and GCMS individually. Moreover, the first guideline on risk evaluation of nanomaterials was developed. By the guideline, imported nanomaterials were determined as low risk while experimental products of Mongolia such as nano silver, SiO_2 and TiO_2 were determined as average risk. The particle sizes of 7 samples (imported 3 disinfectant spray, 2 cleaning solution and 2 experimental products of Mongolia) were measured at the range of 1–110nm, and among them all samples (experimental TiO_2 and SiO_2) produced in Mongolia were tested as mutagenic. It indicates that toxicity could be resulted from method for obtaining nanomaterials besides the particle size. Therefore nanomaterials produced in Mongolia have to be qualified as recommended by the guideline.

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Rapid Diagnosis of Dengue Outbreaks in Resource Limited Facilities

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Dengue is a re-emerging public health problem threatening the tropical developing world, mandating rapid diagnosis in the absence of licensed vaccines or anti-dengue therapy. Regions endemic for dengue and co-endemic viruses are often overwhelmed by the sudden surge of cases during outbreaks wherein dengue is diagnosed and treated on the basis of similar clinical presentation. Related mosquito transmitted co-endemic viral illnesses such as Japanese Encephalitis, West Nile Fever, Yellow Fever and Chikungunya may create considerable interference with diagnosis and management of dengue. While evolving modern healthcare garners accurate diagnosis for patient care and outbreak management, the WHO criteria involve tests exacting in time, expense and effort. A gap exists at the dengue diagnostic front, for which a rapid, sensitive and specific diagnostic methodology was evaluated for dengue outbreaks in resource limited facilities. 100 dengue patients as per WHO Case Definition Criteria for Dengue Fever 2006 as well as matched 100 healthy controls from New Delhi, India were included. All samples were tested by lateral flow immunochromatography (LF-ICT), ELISA and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) by three blinded technicians and results were compared by principal investigator. Diagnostic accuracy indices and Kappa analysis were worked out. Mean age was 33.13 ± 14.85 and male: female ratio was 1.7:1. LF-ICT revealed 58 positive for non-structural antigen-1 (NS1), 42 for IgM and 18 for IgG. RT-PCR revealed 65 positive for dengue. IgM positive by LF-ICT was confirmed by IgM capture ELISA. RT-PCR and NS1 test were compared considering RT-PCR as the gold standard confirmatory test. The sensitivity, specificity, PPV and NPV of NS1 against RT-PCR was 98.31%, 100%, 100% and 99.3%. Observed kappa was 0.99, standard error 0.01, 95% CI 0.96-1 and strength of agreement was perfect (0–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial and 0.81–1 perfect agreement). The sensitivity, specificity, PPV, NPV of IgG against IgM was 44.19%, 100%, 100%, 86.74% and strength of agreement was moderate. No tests were positive in the 100 healthy controls.

Antigen based and molecular tests are a better tool for early diagnosis of dengue. The combined LF-ICT kits are highly sensitive, specific, user-friendly, compact, frugal and thus recommended for use in dengue outbreaks, field conditions and as bed side diagnostic tests. Further studies are required to further assess their utility in prognosis, surveillance and establishment of guidelines for dengue outbreaks.

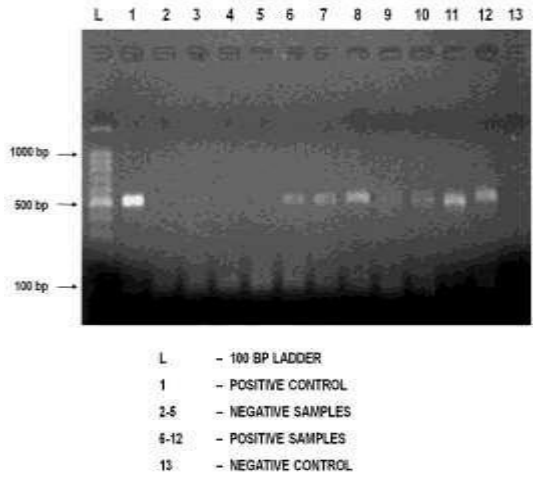

Figure 1. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for dengue showing amplified product at 513 bp utilizing reverse antisense primer (5' TTG-CAC-CAA-CAG-TAC-ATG-TCT-TCA-GGT-TC 3') and forward sense primer (5' TCA-ATA-TGC-TGA-AAC-GCG-CGA-GAA-ACG-G 3')

Posters Presentations

Modulation of Wound Repair *in vitro* in mammalian Cell Monolayers by Poly Functionalised Single Walled Carbon nanotubes

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Carbon nanotubes, a class of nanoparticles have appeared as the wonder materials of the 21st century and have brought new paradigms to diverse fields of modern science. However a majority of carbon nanotubes (CNTs) based nanomaterials are being introduced on the basis of claimed benefits and the ecotoxicological profile of such products remains unknown. With the global annual demand of 3700 metric tons [1], understanding the potential environmental risks and uncertainties associated with the use of CNTs remains a major challenge to the scientific community. Although the general public is less likely to be exposed to toxic concentrations of CNTs in atmosphere, sufficient agitation of functionalized Single Walled Carbon nanotubes (f-SWCNTs) can release fine particles to the peak air borne concentration of 53 $\mu g/m^3$[2] which can be easily inhaled during production or utilization. Therefore risk assessments of CNTs during occupational exposure remain crucial for the safety of workers in nanotechnology. Since the internal milieu of the lungs is in constant contact with the air pollutants, presence of AFSWCNTs in atmosphere can affect the wound repair processes during lung injuries which are common in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). We investigated the effect of acid functionalized SWCNTs (AFSWCNTs) exposure in the migration rates of lung progenitor cells using *in vitro* scratch wound assay. This study identifies that exposure of AFSWCNTs significantly decreases the rate of migration of Human Lung Carcinoma Cells (A549) as well as Murine Alveolar Epithelial Cells (LA4). We also observed that protrusive structures like lamellipodia and filopodia were shorter and fewer in the cells at the wound margin when exposed to AFSWCNTs. These initial findings provide valuable insight that inhalation of AFSWCNTs decreases the migration rates of progenitor stem cells in lungs which is a crucial factor for wound repair during lung injuries.

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Nalidixic Acid Susceptibility Test for Screening *Salmonella* Isolates of Reduced Susceptibility/Higher Minimum Inhibitory Concentration to Ciprofloxacin

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Enteric fever is the major diagnosis among febrile patients in Nepal with yearly increase in nalidixic acid resistance and reduced ciprofloxacin susceptibility among *Salmonella* isolates. This study was carried out to evaluate the validity of nalidixic acid resistance as an indicator of reduced susceptibility of *Salmonella* isolates to ciprofloxacin. In this study, 999 blood specimens collected from suspected enteric fever patients visiting B&B Hospital were processed by standard microbiological techniques. Isolates were identified by biochemical tests and serotyping. Antibiotic susceptibility test was performed by Kirby Bauer disc diffusion method and Clinical and Laboratory Standards Institute (CLSI) recommended interpretive criteria. Minimum inhibitory concentration of ciprofloxacin was determined by agar dilution method. Isolation rate of *Salmonella* species was 6.21%. Among 62 *Salmonella* isolates, 51 were *S.* Typhi, 10 were *S.* Paratyphi A and one isolate was *S.* Paratyphi B. Only one isolate of *S.* Typhi was multi-drug resistant. Resistance to ceftriaxone, cefixime and azithromycin was nil. On disc diffusion test, 55 isolates were resistant to nalidixic acid. Fifty-seven isolates (55 NAR and 2 NAS) were found to have increased (>0.125mg/ml) MIC of ciprofloxacin with the CLSI breakpoints (figure 1). Thus, nalidixic acid resistance showed a predictive value of 100% for ciprofloxacin resistance. Screening with nalidixic acid disc had a sensitivity of 100% and a specificity of 71.43% for the determination of decreased ciprofloxacin susceptibility. Before using ciprofloxacin for the treatment of enteric fever, appropriate identification of *Salmonella* isolates with reduced ciprofloxacin susceptibility is essential to limit the possible treatment failure and further development of highly resistant strains.

Virulence factors and antifungal susceptibility patterns of clinical isolates of Candida species: an experience in a tertiary care hospital in Western Nepal

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Candida remains as harmless commensal or endosymbiont in humans. However, when mucosal barrier is disrupted or host immunity is compromised *Candida* can invade and cause a wide range of infections with significant morbidity and mortality. Number of virulence determinants could contribute to its pathogenicity. Therefore, in the present study, we evaluated the potential of *Candida* clinical isolates in terms of their ability to produce virulence factors which are ascribed to be important pathogenic markers, and also assayed the susceptibility patterns of these isolates against different antifungal agents used in clinical practice.

Isolates were speciated by HiCrome *Candida* differential agar. The virulence factors like biofilm formation, cell surface hydrophobicity, production of DNase, aspartyl proteinase, phospholipase and esterase were measured by earlier improvised methods. The MICs of the isolates against amphotericin B, caspofungin, voriconazole and fluconazole were determined by micro titer broth dilution method following CLSI guidelines.

Out of a total of 71 isolates, 48(67.6%) were *Candida albicans*, 11(15.49%) *C tropicalis*, 09(12.67%) *C glabrata* and 03(4.22%) were *C krusei*. *In vitro* antifungal susceptibility towards commonly used antifungals varied amongst the isolates in terms of the MIC ranges and the geometric mean values. Out of all *Candida* isolates, 43 (60.56%), 44 (61.97%) and 49(69.01%) respectively were positive for proteinase, phospholipase and esterase. None showed DNase activity. However, 77.39% (55/71) isolates were biofilm producers. Majority of those having high cell surface hydrophobicity i.e 48 (67.60%) of 71 tested produced biofilms. Overall, based upon the in vitro characterization of *Candida* isolates, it was observed that significantly higher number of *C albicans* isolates possessed the above mentioned virulence factors as compared to the non-albicans *Candida* species (χ^2=7.53; p<0.01 in respect to phospholipase, χ2=13.23; p<0.001 in respect to proteinase and χ^2=4.51; p<0.05 in respect to esterase.)

Zoonotic Threat of Influenza from Some of the Prevailing Livestock Farming Scenario in Nepal

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Livestock farming is an essential component of agriculture and way of living since the ancient time in Nepal. The raising of different species of animals (cattle, buffalo, sheep, goat or pig) and birds (hen or duck) by a single household is a common practice at rural level. Meanwhile, the some of the aspects of it is likely to pose zoonotic threats of which Influenza can be perceived as the most serious one. A study was undertaken during August, 2015 through field visits, discussions with veterinary microbiologists and review of relevant literature. In a survey undertaken randomly at 128 households (n=128) at Haraiya, Sharadanagar-3 Village Development Committee (VDC), Chitwan, Nepal, it was found that 5.46% (n=7) households were raising both the pig and duck, with their housing being close to each other. The interaction among human, swine and backyard poultry birds may result in the interaction of the influenza viruses which naturally affect them. The major concern is that pig serves as a mixing vessel for exchange of genetic materials between two virus strains (human and avian) and thus resulting a novel and immunologically naive progeny virus. It is because the viral receptors for both human and avian types of influenza virus are present on the respiratory epithelial cells of the swine. The new progeny viruses are capable of causing pandemics and could claim the lives of thousands of people, animals and birds. Thus, it is necessary to disseminate awareness regarding raising either of the two species only. The approaches need to be initiated from the level of government by formulating and implementing rules and regulations regarding raising of multiple livestock species keeping in view the possibilities of emergence and epidemics of viral zoonoses.

Visceral Leishmaniasis in selected communities of Hamer and Benna-Tsemai districts in South West Ethiopia: Sero-epidemiological and Leishmanin Skin Test survey

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Visceral leishmaniasis (VL) is among the neglected tropical diseases. It is known to be endemic in numerous foci in all IGAD member countries. The aim of this study was to determine the prevalence of asymptomatic and Symptomatic VL and also to determine the level of exposure (infection) to Leishmania parasites in the study areas. A community based cross-sectional survey was conducted between 25th of July and August14th of 2013 in 15 selected villages of Hamer and Benna-Tsemai districts which are found in southern Ethiopia, employing multi stage sampling technique. Venous blood was collected for the detection of antibodies using DAT (Direct Agglutination Test) and LST (Leishmanin Skin Test) was also performed to detect the exposure to the parasite. Data was analyzed using SPSS-16 and a P-value of < 0.05 was considered indicative of statistical significance. 1760 individuals 975(55.3%) females and 785(44.7%) males were included. 44.1% of the study subjects were less than 10 years of age .statistically significant variation in the rate of exposure to the parasite was observed in different study sites and age groups. Positive LST response has also shown an increasing trend with age. High DAT positivity was observed in lower age groups. The overall LST and DAT positivity were 8.6 and 1.8% respectively. Asymptomatic VL infection in the area is not negligible and could have a great contribution for anthroponotic transmission of the disease; thus, concerned bodies should take into consideration the implementation of prevention and control strategy for VL. As the area is widely in habited by pastoralists who travel long distance crossing borders in search of food and water for their cattle, IGAD member countries specifically Ethiopia and Kenya should act bilaterally against this deadly disease.

Proportion of *Cryptosporidium* and *Cyclospora* spp. among the School Children of Kathmandu

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Cyclospora cayetanensis and *Cryptosporidium parvum* are the intestinal coccidian protozoans that have emerged as an important cause of parasitic diarrhea among the children living in developing countries. The present study aimed to determine proportion of *Cyclospora* and *Cryptosporidium* among the school children of Kathmandu. Ethical approval for this research was obtained from Institutional review board, Institute of Medicine. A total of five hundred and seven stool samples from students between the age group 3-14 years, studying in 13 different schools of Kathmandu were collected during the study period (May- November, 2014) and processed in Public Health Research Laboratory, Institute of Medicine, Kathmandu, Nepal. A modified acid fast staining technique (Kinyoun's method) was used to detect oocyst of *Cyclospora* and *Cryptosporidium* from the formal-ether concentrated stool samples. *Cyclospora* and *Cryptosporidium* were detected in 3.94% (20/507) and 0.79% (4/507) of stool samples examined, respectively. The prevalence was found to be highest among the students between the age group 3-5 years i.e. 10.15% (13/128) and 3.12% (4/128) for *Cyclospora* and *Cryptosporidium* respectively, peaking during the rainy season (June-August). Infection of the coccidian parasites was found to be significantly associated ($p<0.05$) with presence of livestock at home with prevalence 10.11% (9/89) and 3.37% (3/89) respectively for *Cyclospora* and *Cryptosporidium* and diarrheal symptom among the children with prevalence 10.57 % (11/104) and 2.88 % (3/104) respectively for *Cyclospora* and *Cryptosporidium*. Source of drinking water, mode of water treatment prior to consumption, raw vegetables and fruits consumption, soil contact and family occupational background were found to be associated with the higher rate of infection due to *Cyclospora* and *Cryptosporidium*. This finding confirms a public-health issue with potentially serious consequences whereby, children can be infected through the exposure to an environment contaminated with food and water-borne transmitted oocysts of *Cyclospora* and *Cryptosporidium*.

Bloodstream Infection and Antibiotic Susceptibility Pattern of the Isolates from Patients Visiting Kathmandu Model Hospital

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Bloodstream infection (BSI) is a significant cause of morbidity and mortality in the world. Very limited number of studies on BSIs has necessitated the need of in depth understanding of its cause and implication in Nepal. The aim of this cross-sectional study was to isolate the microorganism responsible to cause BSIs and determine the antibiotic susceptibility pattern of the isolates in patients visiting Kathmandu Model Hospital, Nepal during December 2012 to May 2013. Standard laboratory procedure was used to screen, isolate and identify the bacteria from 1205 patients complaining about BSIs during that period. Antibiotic susceptibility pattern was analyzed by modified Kirby Bauer technique. Data analysis was done using Statistical Package for the Social Sciences version 16.

Out of 1205 blood samples, 186 (15.43%) were culture positive. Most common bacteria isolated were: *Salmonella* spp., *Escherichia coli, Klebsiella pneumoniae* and coagulase-negative staphylococci. Gram-negative bacteria were the predominant causes of BSIs in comparison to gram-positive. *Salmonella* Typhi was isolated in 70.96% cases followed by *Salmonella* Paratyphi A in 16.12%, *Escherichia coli* in 5.37% and *Klebsiella pneumoniae* in 0.53%. Coagulase-negative staphylococcus in 6.98% cases was the only Gram-positive bacteria isolated. There was no significant association between the occurrence of bacterimia to the gender of patients. Gram-negative bacteria were highly sensitive to chloramphenicol with only one isolate (0.57%) resistant towards chloramphenicol. *Salmonella* Typhi showed highest resistance to nalidixic acid 85.6% and ciprofloxacin in 27.24% cases. Gram-positive bacteria showed 100% sensitivity towards chloramphenicol and gentamicin and were least sensitive to amoxicillin.

Yield of Three Consecutive Sputum Specimen in Diagnosis of Pulmonary Tuberculosis in Comparison with Sputum Culture: Active case finding in Bhutanese Refugee living in Nepal

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Pulmonary Tuberculosis (PTB) is commonly diagnosed through sputum smear microscopy examination in Nepal. Previous protocol of conducting three sputum microscopy examinations is reduced to two by National Tuberculosis Programme (NTP) from July 2014. IOM Nepal conducts health assessment for the Resettlement Programme of Bhutanese refugees living in Eastern region of Nepal. Individuals are suspected having PTB on the basis of clinical background, abnormal CXR and positive TST .Suspected PTB cases are further diagnosis by conducting three morning sputum examinations using florescence microscopy, and cultures. This study aim to evaluate the yield of consecutive three days sputum samples in PTB diagnosis. Active surveillance of refugees during Dec 2007-Feb 2014 shows prevalence of PTB as 986 per 100,000 among Bhutanese refugees in Nepal. Total 11,682 refugees were referred for sputum analysis from active screening of 98,903 during Dec 2007 – Dec 2014. Percentage of PTB cases identified by consecutive sputum samples out of either of all three sputum smear positives was calculated to assess additional diagnostic significance. Smear results are compared with culture result of respective day for accuracy of smear analysis. Total 11,682 refugees were tested, 320 (2.74%) were smear positive and 798 (6.83%) culture positive confirmed PTB cases. Diagnostic yield of first smear of sputum with AFB microscopy was 69.7%; incremental yield of 2nd and 3rd samples was 21.3% and 9.1% respectively. Sensitivity and specificity of smear with reference to culture found consistent in each three consecutive samples and in overall (first sample: 31.7% & 99.7%, second: 40.7% & 99.7%, third: 32.2% & 99.7% and overall: 33.1% & 99.5%). We concluded that second and third smear are definitely useful for detecting about 1/5 and 1/10 of the PTB positive cases in active case finding. Similar study would be helpful to see the validity of findings among general population in Nepal.

Study of intestinal parasitic infection among children in Chitwan, Nepal

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Intestinal parasites are the most common cause of parasitic diseases and cause significant morbidity and mortality, particularly in developing countries like Nepal. This study was conducted with the objective to determine the prevalence rate of parasitic infection among children in Chitwan district of central Nepal. A total of 296 stool samples were collected in screw capped container where preserved samples (10% formalin) were tested in Shi-Gan Health Research Laboratory. 3-4 ml stool samples were centrifuged and sedimentation samples examined microscopically under 10x objective followed by 40x objective. Overall, positivity rate was 23.3% (69/296). There is no significance difference between gender, in boys 21.8% (32/147) and girls 24.8% (34/149) (p=0.39). In drinking water, parasitic infection rate in well water was found higher 29.9% (16/55) than tap water 21.9% (53/241) (p=0.261). Infection rate in no drug user was found higher 32.1% (42/131) than drug user 16.0% (27/165) (p=0.002). *Tibeto-Burman* was found highest infection rate of 23.2% (32/138) followed by *Indo-Aryan* 22.1% (29/131) and *Dalit* 29.6% (8/27) (p=0.80). Aged 5-8 years had found highest rate of 26.9% (17/63) followed by 9-12 years 25.15% (42/67) and 13-18 years 15.2% (10/60) (p=0.35). All 10 different parasites were identified.

Taenia spp, *Entamoeba coli* and *Giardia lamblia* was most common (17.4%) followed by *Endolimax nana* (13.0%), *Ascaris lumbricoides* and *Entamoeba histolytica/dispar* (11.6%), *Trichuris trichiura* (4.3%), *Blastocystis hominis* and *Hymenolepsis nana* (2.8%), *and Ancyclostoma doudenale* (1.4%). The burden of parasitic infections among the children, coupled with the poor sanitary conditions in village, should be regarded as an issue of public health priority.

Polyplex nanoparticles as drug delivery system

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Polymer nanoparticles have been extensively studied as the drug, gene and radionuclide carriers in medical applications. They possess advantageous properties such as the possibility of sustained release of the active component, subcellular size and biocompatibility with tissues and cells. Nanoparticles have large surface area and their surface can be modified with photosensitizers, and/or targeting molecules.

We prepared and characterized novel platform based on self-assembled nanoparticles composed of medium and low molecular weight chitosan and alginate. We obtained nanogel by multiple electrostatic interactions between cationic chitosan and anionic alginate and identified key process parameters. The supramolecular complex - polyplex formed by this interaction was used to encapsulate several novel and commercial photosensitizers, including temoporfin (Foscan). Temoporfin encapsulated in the nanogel was stable at the pH of blood plasma (pH 7.4). Particle size distribution was identified by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The diameter of individual particles measured by TEM was approximately 200 nm. Diameters estimated by DLS were slightly biased to higher values. It was found that the size distribution of the nanogel depends on the concentrations of chitosan and alginate stock solutions, the order and ratio of addition, as well as on the pH of the resulting mixture. It appears that samples are homogeneous, although micrographs indicate some (vague, indistinct) core-shell structure. These biodegradable, self-assembling, stable nanoparticles are suitable for photodynamic therapy after topical application or intraperitoneal photodynamic therapy.

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Isolation and Identification of Dermatophytes from Clinical Samples

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Cutaneous fungal infections have been documented worldwide as one of the most frequent human skin infection, which is caused by dermatophytes, yeast and non-dermatophyte molds. Although, dermatophytes are responsible for such fungal infections, the clinical presentation is often confused with other skin disorders. Therefore, the present study was undertaken to isolate and identify etiological agents of dermatophytosis. A one year cross sectional study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College. A total of 100 clinical samples (skin scrapping and nail clipping) received from outpatient department of Dermatology and Venereology, Dr. Prabhakar Kore Hospital and Medical Research Centre, were included in the study. Informed consent and ethical clearance was taken from Department of Dermatology. Samples were processed by Potassium Hydroxide (KOH) preparation for direct microscopy followed by culture in Sabouraud's Dextrose Agar (SDA) with and without antibiotics. Isolates were identified up to species level following conventional methods using standard microbiological guidelines. Chi-Square test was the statistical method used for data analysis. Out of 100 samples processed, 61% cases were found to be culture positive. Dermatophytosis was more common in the age group 21-30 years with manual workers being the most affected. Male: Female ratio was found to be 2.33:1. Most common clinical type of infection was Tinea corporis followed by Tinea cruris. *Trichophyton mentagrophytes* (28, 45.90%) followed by *Trichophyton rubrum* (24, 39.34%) were the most common etiological agent isolated which was different from other findings where *Trichophyton rubrum* was the predominant causative agent. *Trichophyton soudanense* a geographically restricted African dermatophyte was isolated from a farmer with no history of visit to African Country. The studies of the predominating species in the particular region and its relation with various factors affecting its distribution are of considerable importance in implementing proper preventive measures and arresting spread of infection.

Prevalence of intestinal parasitosis and nutritional status among children in orphanages in Nepal

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Intestinal parasitosis and malnutrition are common health problems among children in developing countries like Nepal. The main objective of study was to determine the prevalence of intestinal parasitosis along with nutritional status in orphan children. The prospective study was carried out from October 2013 to June 2014 at National Institute of Tropical Medicine and Public Health Research (NITMPHR), Maharajgunj, Kathmandu. A total of 309 stool samples were collected from Chitwan, Pokhara and Kathmandu Valley. The samples were processed by formalin ether sedimentation technique and were observed under 10X and 40X. The BMI was calculated as per WHO standard.

Over all prevalence of intestinal parasitosis was 20.38% (63/309). Among total parasites, *Entamoeba coli* was found most prevalent (29.41%, 20/68) followed by *Giardia lamblia (*22.06%, 15/68), *Entamoeba histolytica (*17.65%, 12/68), *Trichuris trichiura (*13.24%, 9/68*), Hymenolepis nana (*10.29%, 7/68), *Ascaris lumbricoides (*4.41%, 5/68), and *Endolimax nana* (2.94%, 2/68). 6.34% (4/63) of positive children had co-infection. Females were more infected (21.83%, 31/142) compared to male (19.16%, 32/167). The highest positive rate was found among age group of 5 to10 years children (27.27%, 27//99) than of less than 5 and greater than 10 years ($P<0.05$). Children having soil contact (33.33%, 30/90) were more infected than those having no soil contact (15.06%, 33/219) ($P<0.05$). 16.18 %(50/309) children were found with malnutrition. The parasitosis was higher (25.80%, 8/31), among nutritional status thinness followed by normal (21.23%, 55/259) ($P>0.05$). The present study suggests that the intestinal parasitosis and malnutrition is still common health problems among orphans though they are cared in orphanages. Therefore, proper health awareness and supportive programs should be warranted in national level.

Targeted delivery of nanoparticles to CCR5 expressing cells that can harbor Human Immunodeficiency Virus

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Infectious disease like AIDS has problem of drug toxicity and emergence of viral resistance which limit the effectiveness of many antiretroviral drugs. Therefore developing new therapeutic methods of drug delivery using specific ligands targeting specific receptors that are over expressed on the diseased cells are necessary to treat such diseases. The purpose of this study is to develop a method for ligand/peptide mediated targeting of nanoparticles to CCR5 expressing cells which are HIV harboring cells. In this study poly (d, l-lactic co-glycolic acid) & polyethyleneimine nanoparticles (PLGA-PEI-NPs) were prepared and tagged with peptide ligand (dimerized RANTES) after pegylation with hetero-bifunctional group (NHS-PEG-Mal). Targeting of peptide conjugated NPs (PEG-peptide-NPs) & pegylated NPs (PEG-NPs) to the cells expressing CCR5 (TZM-bl cells) & control cells (U87 cells) showed significantly (<0.05) increased specific targeting of PEG-peptide-NPs in TZM-bl cells as compared to U87 cells. There was also significant ($p<0.05$) efficiency in targeting of PEG-peptide-NPs than PEG-NPs in TZM-bl cells. MTT assay showed no significant change in cell viability with PLGA-PEI-NPs exposure in TZM-bl cells for 72 hours. This study may also be helpful in developing new therapeutic methods for drug delivery where CCR5 are expressed in diseases like basal breast cancer cells, multiple sclerosis, rheumatoid arthritis, GVHD etc.

Knowledge, attitudes and practices (KAP) regarding malaria disease in a highly endemic Jhapa district of Eastern Nepal

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Malaria is one of the endemic vector-borne diseases (VBD) where cases are being reported from 65 districts out of the 75 districts in Nepal. Approximately about 84% (23 million) of the people in Nepal were estimated to be at risk of malaria in 2012, with 4% at high-risk (WHO, 2013).The Government of Nepal has already adopted a long-term malaria elimination strategy with the ambitious vision of a malaria-free Nepal by the year 2026 (EDCD, 2013). Though Nepal has already achieved and exceeded the target set by the Millennium Development Goals (MDGs), universal coverage of malaria control intervention and Roll Back Malaria (RBM) targets of 2010, still malaria remains one of the highly occurring VBD in Nepal. Success of any disease control program depends on community participation. Therefore we were prompted to conduct a study on KAP in a highly endemic Jhapa district of eastern Nepal.

We conducted a community based cross-sectional KAP study in two village development communities i.e. Topagachhi and Korabari VDC of Jhapa using structured questionnaire. Out of 140 participants interviewed, about 80.7% have heard about malaria, Fever and headache (44%) was most commonly known symptoms followed by (25%) mention shivering and sweating. Only 46% perceived mosquito bite transmit the diseases while (66%) mention stagnant water as a breeding place for malaria vector. About 72.5% of the respondents use bed nets and 7% spray chemical insecticides. Government intervention program for control of malaria was known to 65.5%. Whereas only 41.6% preferred public health sector for treatment and 38.0% preferred to go to traditional healers.

We concluded that though malaria was major health problem in Jhapa district since long time the participants still have poor knowledge and practice about the disease. Therefore, large coverage of health educational campaigns should be implemented involving community participation for a successful elimination program.

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Characterization of *Vibrio parahaemolyticus* isolated from shrimps and shrimp fields located in the Satkhira coastal area, Bangladesh

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This study was carried out to isolate and characterize *Vibrio parahaemolyticus* isolates from shrimps and shrimp fields located in the Satkhira coastal area. The contamination of shrimp with pathogenic *V. parahaemolyticus* could be a threat for the shrimp export sector which is the second largest export earner for Bangladesh. Thirty four *V. parahaemolyticus* were isolated and confirmed by standard biochemical, serological and molecular techniques. All 34 *V. parahaemolyticus* isolates belonged to 10 serogroups (O10:KUT, O1:K25, OUT:K61, O11:K33, O2:K28, O1:K33, O1:KUT, O5:KUT, O11:KUT and O8:KUT) confirmed by duplicate testing. Along with the occurrence of thermostable direct haemolysin (TDH) and TDH-related haemolysin (TRH), the incidence of two sets of gene clusters that encode the type 3 secretion system apparatus (T3SS1 & T3SS2α) which contribute in pathogenicity of *V. Parahaemolyticus* were also investigated. All *V. parahaemolyticus* isolates were found to be negative for both *tdh* and *trh* genes, which were confirmed by PCR. T3SS1 was found to be present in all *V. parahaemolyticus* strains as confirmed by the PCR. All these environmental *V. parahaemolyticus* strains were found to be negative for T3SS2α which confirmed that T3SS2α is not associated with non-pathogenic environmental strains. We also phenotypically characterized the environmental isolates for the enterotoxicity test, which showed no fluid accumulation in rabbit ileal loop assay. All the above mentioned tests were done twice and the variances were less than 5 percent. All these findings clearly indicate that the *V. parahaemolyticus* organisms, which are associated with the shrimp and shrimp fields of the coastal area of Satkhira are non-pathogenic in nature thus would not be a threat for shrimp export sector as long as the concerned bacterium is *V. parahaemolyticus.*

Prevalence of multidrug resistant (MDR) and extended spectrum beta-lactamase (ESBL) producing bacteria in various clinical isolates

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Antibiotic resistance has been recognized as one of the major global public health threat. The extensive and haphazard use of antibiotics has raised multidrug resistant (MDR) organisms mostly in hospital settings. One of the mechanisms of development of MDR by microbes is through production of extended-spectrum beta-lactamases (ESBLs). ESBLs are the major defence of Gram negative bacteria against beta-lactam agents which are most frequently prescribed antibiotics worldwide. In Nepal, it is a real challenge to control ESBL producing bacteria due to its rapid emergence. The main objective of this study was to determine the prevalence of multidrug resistant and extended spectrum beta-lactamase producing bacteria in various clinical isolates obtained from August 2013 to June 2014 at Annapurna Neurological Institute and Allied Sciences. During working period, about 304 samples including urine, sputum, blood, pus, swabs, catheterized tube cultures, tracheal aspirates, CSF, body fluids and others were processed. Antibiotic susceptibility testing was performed by Kirby-Bauer disk diffusion method and ESBL detection was done by combined disk assay using cefotaxime and cefotaxime/clavulanic acid and ceftazidime and ceftazidime/clavulanic acid following CLSI guidelines. Out of total 161 identified isolates, Gram-negative bacteria constituted 81.9% (132/161) and Gram-positive bacteria constituted 18.0% (29/161). *Pseudomonas* spp. was predominant among Gram-negative bacteria with 29.5% (39/132) and *Staphylococcus aureus* was predominant among Gram-positive bacteria with 58.6% (17/29). Out of 161 isolates, 115 (71.4%) and 54 (33.5%) isolates were MDR and ESBL producers respectively. Among ESBL producers, *Pseudomonas* spp. and *Escherichia coli* were predominant bacteria with 24.1% (13/54) and 24.1% (13/54) respectively. Similarly, antibiotic susceptibility pattern revealed that bacteria were mostly resistant among antibiotics like ciprofloxacin, cefixime, cefotaxime, amoxicillin and so on. Thus, the stringent antibiotic stewardship program is mandatory in developing countries like Nepal to control the emergence of multidrug resistant strains.

Key words: multidrug resistance, extended spectrum beta-lactamase, antibiotic susceptibility testing

Methicillin resistant *Staphylococcus aureus* (MRSA) at B P Koirala Institute of Health Sciences (BPKIHS), Dharan: A laboratory perspective

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Methicillin Resistant *S. aureus* (MRSA) is a strain of *S. aureus,* which are resistant to all β-lactam antibiotics including cephalosporin, carbapenems but may be susceptible to recent generation of cephalosporin (eg-ceftaroline). Threat of MRSA is continually increasing. Mostly, MRSAs are determined by phenotypic characterization of clinical isolates but genetic method based on *mecA*-PCR is uncommon in routine clinical settings. In this context, our study was undertaken to evaluate the performance of *mecA*-PCR and to compare its result with another Clinical and Laboratory Standards Institute (CLSI) recommended cefoxitin disc diffusion assay in the panel of 65 well characterized *S. aureus* obtained from various clinical specimens over the period of one month in microbiology laboratory of BPKIHS, Dharan, Nepal. Detail analysis showed that 37% of MRSA were estimated by disc diffusion assay versus 58.3% of MRSA by PCR. Indeed, our PCR documented higher rates of MRSA in BPKIHS. However, this result should be considered as the first step for the exploration of *mecA* gene but their further verification in larger number of samples are recommended not only for real estimation of MRSA but also for molecular tracking to control health care associated infection in such type of tertiary care center.

Antibiotic susceptibility patterns of *Vibrio cholerae* isolates in Kathmandu in 2015

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Cholera is caused by the bacterium *Vibrio cholerae* and is spread mainly through contaminated water. Patient can die within several hours due to severe dehydration if treatment is not administered promptly and accurately. Antimicrobial resistance patterns can change in the same place over a period of time. Kathmandu valley has been witnessing outbreak of cholera over the past several years affecting hundreds of people, particularly young adults. Appropriate antibiotics can reduce the volume of diarrhea and shorten the duration of hospital stay. Thus, this study was undertaken to understand the patterns of antimicrobial resistance to ensure appropriate antibiotics to be used in patients with cholera infections. A total of 431 patients with acute diarrhea visiting to the Sukraraj Tropical and Infectious Disease Hospital were enrolled in the study. Stool specimens were cultured on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar by overnight at 37C and fermenting yellow shiny colonies were subjected to biochemical identification through TSI, SIM, urease, citrate, oxidase and string test. Serological confirmation and serotyping of *Vibrio choleare* was done using specific antisera. Antibiotic sensitivity pattern was done using standard disc diffusion technique as per the guidelines of Clinical Laboratory Standard Institute (CLSI).

A total of 78 *Vibrio cholerae* were isolated of which 77 were serotype Ogawa and the remaining one was of Inawa serotype. All isolates were sensitive towards tetracycline, norfloxacin and azithromycin. Sensitivity to ciprofloxacin was found to be intermediate. All the strains were resistance to ampicillin, co-trimoxazole and nalidixic. Although most of patients with cholera infection were unconscious at admission, rehydration therapy along with selected antibiotic therapy has shortened the hospital stay on an average of 2 days. Continued monitoring of antimicrobial susceptibility patterns is needed in order to guide the accurate choice of therapy and in developing evidence-based National Guidelines for cholera treatment.

Green Synthesis of Silver nanoparticles using plant extracts and evaluation of their antibacterial activity

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The synthesis of nanoparticles from biological process is evolving a new era of research interests in nanotechnology. Plant mediated synthesis of nanoparticles is a Green Chemistry approach that interconnects nanotechnology and biotechnology. The present work describes a cost effective and environment friendly technique for green synthesis of silver nanoparticles (Ag NPs) from aqueous $AgNO_3$ solution through the leaves extract of *Cannabis sativa*, *Nyctanthes arbor-tristis*, *Solanum nigrum*, *Prunus persica* and *Taraxcum officinale* as a reducing as well as capping agents and analyzed them by UV-Visible Spectroscopy, Diffuse Reflectance UV-Vis Spectroscopy (DRS), X-Ray Diffraction (XRD), Attenuated Total Reflectance Fourier Transform-Infrared Spectroscopy (ATR-FTIR), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The synthesis of silver nanoparticles was confirmed by monitoring change in color from light yellow to dark brown, and by evaluating the Surface Plasmon absorption bands. The TEM reveals that the Ag NPs were successfully synthesized through *Taraxcum officinale* which had mean size of 33.68 ± 10.91 nm (centrifugation), 16.85 ± 7.30 nm (ethanol precipitation) and through *Nyctanthes arbor-tristis* which had mean size of 14.62 ± 5.03 nm (ethanol precipitation). The present study suggests *Nyctanthes arbor-tristis* as a good source for generation of more uniform silver nanoparticles in comparison to *Taraxcum officinale*. The present study also suggests that ethanol precipitation as a better option for purification and separation of silver nanoparticles in comparison to centrifugation. The antibacterial effects of these nanoparticles were studied against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris and Staphylococcus aureus*. The present study indicates that these silver nanoparticles have low antibacterial activity.

Keywords: Silver Nanoparticles, Green synthesis, Antibacterial, Plant extracts.

Status of antibiotic resistance in Gram Negative clinical isolates in Kathmandu Valley

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Emergence of antibiotic resistance in bacteria has been a serious global threat in both health care setting and community which is attributed to misuse/abuse of antibiotics. This has been apprised in Nepal as well. Therefore, present status of antibiotic resistant pattern and MDR among clinical isolates was studied. A total of 111 gram-negative bacteria (all bacilli) isolated from 1,224 different clinical samples (urine: 736, blood: 411, pus: 22 and others: 55) investigated during May-August, 2014 were included in this study. The organisms isolated were *E. coli* (61/111; 54.9%), *Salmonella* spp (16/111;14.4%), *Klebsiella* spp (13/111; 11.7%), *Proteus* spp (9/111; 8.1%), *Citrobacter* spp (5/111; 4.5%), *Pseudomonas* spp (3/111; 2.7%), *Enterobacter* spp (2/111; 1.8%), *Serratia* spp (1/111;0.9%) and *Shigella* spp (1/111;0.9%). These bacteria were further subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion method with 11 different antibiotics. The overall prevalence of multiple antibiotic (drug) resistance was found to be 50.4% (67/111). Individual bacterial multi antibiotic (drug) resistant pattern was as follows: *E. coli* 73.7% (45/61)*; Kleibseilla* spp 30.7% (4/13)*; Salmonella* spp 31.3% (5/16) and *Proteus* spp 88.8% (8/9). Among the drugs used, ofloxacin, cefexime and ceftriaxone were effective against *E. coli* whereas amoxycilin was least effective. *Salmonella* spp were sensitive to ofloxacin, cefixime, azithromycin and chloramphenicol but resistant to nalidixic acid. Similar results were observed in other isolated bacteria. The results of this study suggested a need for continued surveillance of antimicrobial resistance among pathogens and stoppage of misuse and abuse of antibiotics.

Balantidiasis: An encounter with a rare parasitic infection

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Balantidium coli, an uncommon ciliate protozoan parasite, is known to cause Balantidiasis. *B. coli* is found to have a global distribution with a prevalence of about 0.02 – 1%, and are most commonly associated with pigs. The infection in human is generally a rare encounter, acquired by ingestion of food and water contaminated by cysts causing bloody diarrhoea similar to that of amoebic dysentery. Here, we report the detection of the rare parasite, *B. coli* in a 37- year- old male with tuberculosis and presenting with fever, anorexia, mild abdominal pain and episodes of loose stools for a week. Freshly passed loose stool sample from the patient was received in the parasitology section, Department of Microbiology, JIPMER. The stool specimen was watery and contained blood and mucus. Routine stool microscopy and Trichrome staining was performed immediately after receiving the sample, which showed the presence of motile trophozoites about 65 µm in length and 30 µm in width and cysts of varying sizes ranging from 15- 30 µm in diameter. The active ciliated trophozoites were identified as *B. coli* and the cysts were identified as that of *Entamoeba coli*. Further work up on the patient showed no extra-intestinal manifestations like other necrotising lung infections, peritoneal spread or any genito-urinary lesions. Astoundingly, *B. coli* is the largest ciliate protozoan and only member of the family *Balantidiidae* that is pathogenic to human. This study is one among the few in the global scale, which has reported the detection of *B. coli* in human. Considering the pathogenic potential of the parasite in malnourished population and immuno-compromised individuals, detection of *B. coli* should be regarded as essential in all cases of dysentery. This will aid in better understanding of the parasite, its distribution and epidemiology.

Study of under-utilized plants of Nepal for therapeutic purposes

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Nepal, despite being rich in biodiversity, has not been able to utilize its huge resources of medicinal plants. Phytochemicals can be investigated for therapeutic purposes. The aim of the present work was to explore the medicinal values of under-utilized plants. Ten species (*Lygodium japonicum, Randia tertasperma, Sapindus mukorossi, Punica granatum, Catharanthus roseus, Camelia kisii, Albizia chinensis, Eophorbia pulcherrima, Clerodendrum japonicum, Ficus religiosa*) of such plants were selected for the study. Methanolic extracts were used for the phytochemical analysis. The total phenolic and flavonoid content were estimated spectrophotometrically using Folin Ciocalteu and aluminium chloride colorimetric methods respectively. The highest and lowest amount of flavonoid was shown by *Ficus religiosa* (57.49±0.60mg of QE/g) and *Albizia chinensis* (22.45±0.18 mg of QE/g) respectively. The highest and lowest phenolic content was shown by *Punica granatum* (228.73±10.56 mg of GA/g) and *Euphorbia pulcherrima* (17.37±2.01mg of GA/g) respectively. The antimicrobial activity was tested against Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria and fungi (*S. cerevisiae and P. pastoris*). *Punica granatum* showed antibacterial activity against Gram positive bacteria while the extracts were ineffective against Gram negative bacteria. Antifungal activity was observed only from *Camellia kisii*. DPPH (2, 2-diphenyl-1-picryl hydrazyl) assay was carried out to evaluate the antioxidant activity. *Ficus religiosa* showed the highest antioxidant activity with IC_{50} value of (13.87±0.53 µg/ml) in comparison to ascorbic acid. The preliminary test for compounds with anti-cancerous activity was performed using brine shrimp lethality assay at various concentration. Three extracts (*Camellia kisii, Albizia chnensis,* and *Euphorbia pulcherrima*) showed high activity at 100ppm, one more (*Clerodendrum japonicum*) extract showed at 200ppm while all the other extracts showed high activity at 500ppm. Thus, selected under-utilized plants showed presence of bioactive compounds which can be further identified and isolated for therapeutic activity.

Bacterial Infections and emerging resistance in renal transplant recipients

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Renal transplantation is frequently complicated by bacterial infections in the scenario of immunosuppression, altered metabolism and interventions resulting in prolonged morbidity. Subdued clinical presentation, antimicrobial resistance and toxicity tend to jeopardize the outcome of transplantation. This study highlights infections as well as antibiograms in Renal-Transplant-Recipients (RTR) in comparison with nephrology-ward-in-patients (NIP). 130 RTR and 160 NIP were included. Pre-transplant evaluation included clinico-demographic parameters, transplant indications, donor profile, HLA matching, infection screen and vaccination. Post-transplant immunosuppression, cotrimoxazole prophylaxis, infection profile and antibiograms were noted. Infections and antibiograms were compared. RTR included 70.8% males and 29.2% females between 15-50 years. Common indications for transplants included Chronic glomerulonephritis (58.5%) and Chronic interstitial nephritis (21.5%). Mothers (38.5%) outnumbered other donors. Thymoglobulin, Basiliximab and Daclizumab were used for induction while Mycophenolate mofetil, Tacrolimus and Prednisolone for maintenance. Post-surgical infections in the first week post- transplant were most commonly encountered. 81.5% urinary and 27.7 % blood stream infections with 12.3% polymicrobial, 6.2% prolonged and 1.5% disseminated infections were seen. Bacteremia secondary to urinary infections was seen in 23.1% cases. *E. coli* (29.9%), *Pseudomonas aeruginosa* (17.9%), *Acinetobacter baumanii* (12.5%), *Klebsiella pneumoniae* (7.14%) and *Staphylococcus aureus* (6.7%) were isolated. The infection profile of NIP included 27.4% *Pseudomonas aeruginosa,* 18.9% *Acinetobacter baumanii* and 15.1% *E. coli.* Comparative resistogram of RTR and NIP included 88.4% and 63.2% β-lactamase producing Gram positive bacteria with 80% and 60% MRSA (p=0.04); 36.9% and 31.8% ESBL producing Gram negative bacteria respectively. Transplant programs, even with established protocols, remain prone to frequent, disseminated, simultaneous polymicrobial bacterial infections with emerging multiresistant organisms complicating transplant outcome. Urinary infections with multiresistant *E. coli* and *Klebsiella pneumonaie* can lead to bacteremia. A higher degree of multiresistance may exist in transplant recipients mounting a therapeutic challenge. Multiresistant organisms harboured by transplant recipients may contribute to nosocomial hazard which emphasizes infection control measures.

CLSI-ESBL phenotypic confirmatory test in urine isolates

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Various bacterial species belonging to family Enterobacteriaceae produces extended spectrum beta lactamase (ESBL) enzyme and are highly resistant to many penicillins, cephalosporins and aztreonam antibiotics. *E. coli* and *Klebsiella* are two most important ESBLs producing bacteria. *E. coli* cause urinary tract infections which can develop to more severe infections like blood poisoning, that can be life threatening. Increased resistivity against several groups of antibiotics makes these infections more complicated to treat. In this study, 323 urine samples collected from different hospitals of Karachi were inoculated on MacConkey and Cystine Lactose Electrolyte Deficient (CLED) agar medium. Isolates were identified using cultural characteristics, microscopy and biochemical testing. Antimicrobial susceptibility was tested by the disk diffusion method using Mueller-Hinton agar. Antimicrobial agents tested were ampicillin, amoxicillin-clavulanic acid, aztreonam, cefuroxime, ceftriaxone, ceftazidime, cefipime, piperacillin-tazobactam, meropenem, gentamycin, ciprofloxacin, cotrimoxazole, nitroforentine and fosfomycin. The CLSI-ESBL phenotypic confirmatory test with ampicillin, ceftriaxone, and amoxicillin-clavulanic acid was performed. A minimum of 5 mm increase in the zone of diameter of third-generation cephalosporins, tested in combination with amoxiclav versus its zone when tested alone, was considered indicative of ESBL production. All the isolates were susceptible to imipenum (10 µg/disk). *E. coli* ATCC 25922 was used as ESBL-negative and *K. pneumoniae* 700603 was used as ESBL-positive reference strain. Results showed 79 (24%) ESBL producing organisms out of 323 samples. Within the ESBL susceptible samples, 32% belonged to males and 68% to females in which *E. coli* 27%, 11% *K. pneumonia* and *K. oxytoca* were 23% were ESBL positive. Data were analyzed by SPSS version 17. Frequencies and percentages were computed for categorical variables like microorganisms, antimicrobial sensitivities, sex etc. *E. coli* showed simultaneous resistance to third-generation cephalosporins, aminoglycosides, and fluoroquinolones. A genetic investigation on these isolates should be performed in order to confirm the presence of plasmid mediated TEM, SHV, CTX-M beta lactamases gene, are the most common in ESBL producing organisms.

Seroprevalence of Leptospirosis among the patients presenting with febrile illness in Bidar, Karnataka

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Leptospirosis is a bacterial zoonotic disease caused by Spirochete *Leptospira interrogans* complex which is prevalent all over the world. Humans become infected through contact with contaminated animal urine, tissues or water. The clinical presentation is difficult to distinguish it from Dengue, Malaria, Influenza and many other diseases characterized by fever, headache and myalgia. Leptospirosis is known to be endemic in India since the early 20th century. Recent reports include the 2007 outbreak in Raichur of Karnataka, where 1516 cases were treated for Leptospirosis. Bidar is a town in the North eastern Karnataka of India with population of 15.02 lakhs. 77.04% of the population reside in rural areas with poor level of sanitation and contaminated environment. This study was done to check Seroprevalence of leptospirosis among Bidar population attending Bidar Institute of Medical Sciences (BRIMS) Teaching Hospital with febrile illness. An independent study was conducted in the Referral Laboratory Network, Integrated Disease Surveillance project, Department of Microbiology, BRIMS Bidar in April 2015. 150 serum samples which were tested negative for Widal, Dengue, Malaria, Brucella included in the study. These samples were tested for Leptospira Microplate Immunoglobulin M (IgM)-Enzyme Linked Immunosorbent Assay (ELISA). Among 150 samples, 12% samples were positive with 33.3% in Male and 66.66% in female patients. Highest infectivity rate was found among age group between 30-40 (38.88%). Statistical analysis was used to calculate percentage and proportions. Our study findings were surprising as 12% of cases left undiagnosed for Leptospirosis. In conclusion, this is an alarming sign for the clinicians of this region to include Leptospirosis in the differential diagnosis of febrile patients. Further, it is recommended the government to make available rapid testing facilities for early diagnosis of Leptospirosis in Taluk/District Hospitals and Microscopic agglutination test (MAT) in Microbiology Department of Government Medical College in the district.

Study of virulence and predisposing factors in Quinolone resistant uropathogenic *E. coli*

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E.coli is the commonest pathogen encountered in urinary tract infections (UTI), where fluoroquinolones are frequently prescribed for empirical treatment. However emergence of quinolone resistant *E. coli* has been reported by several authors. Presences of multiple virulence factors as well as predisposing factors are correlated with fluoroquinolone resistance. This observational cross sectional study was conducted in the Department of Microbiology, S.C.B. Medical College, Cuttack, India, from July 2011 to May 2012. Ninety eight (98) *E. coli* [40%; 95 % C.I - 96.25% -99.75 %] were isolated from urine samples of 245 clinically diagnosed cases of UTI. Predisposing factors like gender, age, catheterization and history of previous quinolone intake were collected during sample collection. Fifty-six (56) isolates were confirmed to be quinolone resistant (MIC > 1 μgm/ml) by agar dilution method. All the 98 *E.coli* isolates were subjected for virulence factor study such as hemolysin production in 5% sheep blood agar, serum resistance and biofilm production by microtitre plate method (Wakimoto *et al* 2004). Quinolone resistance was noticed in 62.5% female patients, 60.7 % in sexually active age groups (21-45 years), 32% of catheterized patients and 57% of UTI having history of previous intake of quinolones. Hemolysin, serum resistance and biofilm production were detected in 76.7%, 67.8% & 50% of the quinolone resistant isolates respectively. 23.2% of isolates showed all the three virulence factors in combination. Among the predisposing factors- females, catheterized patients and history of previously intake of quinolone were significantly ($p < 0.05$) associated with quinolone resistant uropathogenic *E.coli* in UTI patients. Hence empirical use of quinolones should be discouraged.

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Prevalence of intestinal parasitic infections, related risk factors and possible impact on nutritional status among public school children in Kathmandu

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Intestinal parasitic infections are the major public health problem in Nepal. This cross-sectional study was conducted to determine the prevalence of intestinal parasitic infection and its association with different risk factors and nutritional parameters among school children. Stool and blood samples were obtained from 311 primary level school children of a public school in Kathmandu, Nepal from June to September 2014. Samples were transported to Shi-Gan International College of Science and Technology (SICOST) laboratory for further investigation of parasites in stool and hemoglobin level in blood. Stool samples were processed for microscopy by formal-ether concentration technique and hemoglobin was estimated by cyanmethemoglobin method. Anthropometric data related to their demography, personal hygiene, socio-economic condition were collected. Statistical analysis was done by using SPSS 20.0. The results showed that the prevalence of intestinal parasitic infection was 19.6% with higher rate of protozoan parasites than helminthes. *Giardia intestinalis* was the most common protozoan parasite followed by *Entamoeba coli*, *Endolimax nana* and *E. histolytica*. Among the helminthes *Trichuirs trichiura* was highest followed by *Ascaris lumbricoides* and *Hymenolepis nana.* Prevalence of the intestinal parasitosis was significantly higher among the children eating street foods ($p<0.05$). No statistically significant association was observed with respect to gender, age, parents' literacy, playing on soil, use of antihelminthic drug, source of drinking water and treatment of drinking water. About 26.4%, 18.9% and 7.4% children were stunted, underweight and wasted, respectively. No significant association was seen between prevalence of malnutrition and prevalence of infection. About 18.6% of the children were anemic. No significant association was seen between the prevalence of infection and anemia. The prevalence of intestinal parasitic infection and malnutrition are not negligible among school children. Further study should be done on the quality of the street foods.

Prevalence of Nosocomial Infection by Methicillin Resistant *Staphylococcus aureus* in a Health Care Center

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Methicillin resistant *Staphylococcus aureus* (MRSA) infection is a major cause of nosocomial infections worldwide. MRSA can cause an outbreak as it can be transmitted easily from one person to another. Nepal is a developing country with relatively less developed health care infrastructures and clinical practices. There are very few data on prevalence of nosocomial MRSA in Nepal. This study was carried out to determine the prevalence of nosocomial infections by MRSA at Alka Hospital, one of the leading private hospitals in Lalitpur from November, 012 to April, 013. Three hundred and sixteen samples were collected from the patients who stayed for more than 48 hours in hospital and was further processed with standard microbiological methods. Samples included wound & soft tissue swab, pus, blood, sputum, urine and devices. Sample exclusion criteria included patients who had intranasal mupirocin or polysporin and oral antimicrobials for eradication of MRSA within past fourteen days. Sample inclusion criteria included use of invasive procedures, wound and soft tissue infections and/or referred by medical officer. Mannitol Salt Agar was used as selective medium for *Staphylococcus aureus*. The antimicrobial susceptibility test was performed by Kirby-Bauer disc diffusion method as per Clinical Laboratory Standard Institute (CLSI, 2007) guidelines. Cefoxitin was used to differentiate MRSA from MSSA. Growth was seen only in 79 out of 316 samples collected and *S. aureus* (37%) was found to be dominant organism followed by *Escherichia coli, Acinetobacter spp, Pseudomonas spp, Klebsiella spp* respectively. Among 29 *S. aureus* isolated, 27.6% *S. aureus* was found to be methicillin resistant. The maximum number of *S. aureus* was isolated from the ICU ward. The number of MRSA and MSSA infection increased with the age of patients ($p<0.05$). The antibiogram of MRSA and MSSA showed great variance. The highest number of *S. aureus* was resistant to Amoxicillin (62.06%) and least resistant to Gentamycin (24.1%). The prevalence of nosocomial infection by MRSA highlights the need of regular surveillance in order to control MRSA.

Prevalence of Multidrug-Resistant *Pseudomonas aeruginosa* in Clinical Samples

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Pseudomonas aeruginosa is an important pathogen, frequently isolated from hospital settings and the isolation of multidrug-resistant (MDR) strains has occurred as a major problem. The study was conducted in Nepal Armed Police Force hospital, Balambu, Kathmandu from March 2013 to September 2013 with the objectives to determine the prevalence of multidrug-resistant *P. aeruginosa* in clinical samples. The collected specimens were identified by standard microbiological method and antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method. Among the total of 523 clinical specimens, 25 (14.5%) samples showed growth of *P. aeruginosa*. A total of 14(56%) positive specimens were obtained from the indoor patients while remaining 11(44%) specimens were from outdoor patients. *P. aeruginosa* isolated from inpatient samples demonstrated higher resistance to the tested antibiotics than that from the outpatient samples. The maximum resistance was shown towards colistin followed by ciprofloxacin. Among these, 4 (16%) *P. aeruginosa* were found to be multidrug- resistant. The occurrence of multidrug-resistant organism poses a serious threat resulting in treatment failure in infected patients. Regular monitoring and an effective hospital infection control policy are necessary to control the rise of multidrug-resistance.

Diagnostic Yield of Third Sample in Consecutive Three Days' Sputum Smear Testing for Pulmonary Tuberculosis: Finding from Active Surveillance among Immigrants from Nepal

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Sputum smear microscopy is widely used method for pulmonary tuberculosis (PTB) diagnosis in Nepal. NTP Nepal used to conduct sputum smear microscopy of three samples which is reduced to two from July 2014. Available literatures have no uniform recommendation for third sputum tests. WHO estimates prevalence of all forms of Tuberculosis in Nepal as 211 per 100,000 population (WHO, 2014). IOM is conducting 3 sputum smear examinations using florescence microscopy, and solid and liquid culture for diagnosis of individuals identified as possibly having PTB based on medical history, physical examination, CXR and TST. Active surveillance of emigrants bound to countries like USA, UK and Australia during Aug 2010-Feb 2014 shows prevalence of PTB as 393 per 100,000 in Nepal. This analysis assesses yield of third sputum microscopy of 2,951 migrants identified as possibly having PTB disease from active surveillance of 31,457 emigrants in IOM Nepal during Aug 2010 – Dec 2014. Yield of each consecutive sputum sample is assessed as percentage of total sputum smear positives from either of all three sputum samples. Accuracy of sputum smear results is assessed by comparing with findings from cultures of respective samples. Out of 2,951 emigrants tested 79 (2.7%) were smear positive and 199 (6.7%) were culture confirmed PTB cases. First sputum smear examination identified 59.5% of all smear positives, second specimen added 27.8% and third 12.7%. Sensitivity and specificity of all three consecutive samples in reference to culture were consistent with each other and with overall (first sample: 27.3% & 99.7%, second: 32.2% & 99.8%, third: 27.3% & 99.7% and overall: 31.2% & 99.4%). Among emigrants from Nepal, third sputum sample is useful in detecting more than 12% of PTB cases which could have otherwise missed. The situation may be similar in the general population but further study would be needed.

Looking toward the possibility of the canonical role of APOBEC3B in human diseases

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The APOBEC (Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) family of proteins comprises of a group of cytidine deaminases that are able to edit DNA and/or RNA sequences. The APOBEC3 family in humans consists of: APOBEC3A (A3A), APOBEC3B (A3B), APOBEC3C (A3C), APOBEC3DE (A3D), APOBEC3F (A3F), APOBEC3G (A3G) and APOBEC3H (AH). The subcellular localization of APOBEC3 proteins can be nuclear or cytoplasmic. APOBEC proteins have diverse role in immunity, retroviral restriction and antibody maturation. Recent reports have suggested a role of APOBEC3B in cancer. It has been shown that expression of APOBEC3B is highly up regulated in all forms of cancer. APOBEC3B is highly expressed in the human spleen and shows role in immune clearance of the parasites. It has been found that A3B localizes to nucleus and alters the cell cycle. We tested the expression profile of A3B in various cell lines. It is highly overexpressed in human T cell line HuTR5 and has little or no expression in Lung cancer cell line LA-4. We aimed at looking the reason(s) how A3B is playing role in cancer and different diseases. We performed coimmunoprecipitation experiment to pull down A3B followed by LC/MS. Data generated is currently being analyzed to understand the functional role of proteins precipitated along with A3B.

Intestinal parasitosis among school children in different geographical areas in Nepal

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Intestinal parasitosis is common worldwide particularly in areas with poor sanitation and poverty, and there is variation in prevalence with geographical locations. In this paper, findings of a cross-sectional study done among the school children of three different geographical areas area presented. A total 713 stool samples collected from school children [Mountain (Mustang District): n=208; Hill (Gorkha District): n= 222 and Plain (Saptari District): n=283) areas] in 2015 were included. Samples collected in screw capped plastic container and fixed with 10% formal-saline were transported to Shi-Gan Int'l College of Science and Technology (SICOST) Laboratory in Kathmandu. Basic information on basic hygiene and sanitation were collected. Parasites in stool samples were detected by formal-ether sedimentation technique. The statistical analysis was done by SPSS 20.0. The overall intestinal parasitosis was 24.5% (175/713) with lowest in Mountain region (16.3%; 34/208) and highest in Terai (plain) (32.5%; 92/283). Among the parasites detected, protozoan parasites were dominant (94.2%; 165/175) over helminthes (5.7%; 10/175)) and this trend was true in all three regions. *Giardia* was the dominant followed by *Entamoeba coli* and others. Girls were infected more than boys but without significant difference. The higher prevalence in Terai area appeared to be due to flat land related sanitary condition. Dominance of protozoan parasite over helminthes must be associated with deworming program that does not affect the protozoa. Present findings, therefore, indicated that there is difference in prevalence of intestinal parasitosis with geography of Nepal.

Dynamics of T cell proliferation using Ki67 antigen staining in HIV-1 infection

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Progressive T cell depletion is the cardinal feature of HIV-1 infection however, the exact cause of this altered dynamics in T cell compartment remains elusive till date. In the present longitudinal study, proliferation frequency of different T cell subsets was investigated among HIV-1 infected individuals and healthy controls. This study not only enhances our knowledge of HIV immunopathogenesis but also provides novel information about the dynamics of naïve and memory CD4+ and CD8+ T cell proliferation. 10 healthy and 20 HIV-1 infected individuals were enrolled for the study. Expression of Ki67 nuclear antigen was examined on HIV specific T cell subsets in peripheral blood lymphocytes using Intracellular Ki67 Staining. The median proliferating frequency of CD8 CM ($p=0.02$) and naive ($p=0.006$) cells was significantly higher in HIV infected subjects compared to healthy controls. At baseline, we observed that central memory subset of both CD4+ and CD8+ T cells were more proliferative followed by naïve cell subset in untreated HIV infected individuals (CD4+ CM: $p= 0.9256$; naïve: $p = 0.5279$; CD8+ CM: $p= 0.081$; naïve: $p = 0.1169$) however, a decline was observed in the proliferation of naïve, CM, EM and TEMRA subsets after six months of follow up in both the compartments. We observed a negative correlation of baseline viral load with the Ki67 expressing T cell subsets and positive correlation at follow-up. Our findings clearly indicate that the unremitting rounds of memory T cell expansion exert a negative pressure on the naïve T cell pool resulting in dysregulated T cell balance. This result in exhaustion of the naïve T cell pool and put strain on the maintenance of the resting memory T cell pool.

Screening, isolation and multiple-host-range analysis of bacteriophages isolated from rivers of Kathmandu valley against various multi-drug resistant bacteria

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Bacteriophages or simply phages are viruses that infect bacteria. They are the most abundant and ubiquitous organism on earth. Antibiotic resistance has been one of the greatest threats to modern medical achievement because of non-regulated use of antibiotics. Among all alternatives sought to tackle antibiotic-crisis, phages have been used longest in clinics (Reardon, 2015). Since lytic phages can naturally infect and lyse specific bacteria but leave animal/plant cell unscathed, they hold immense possibility in fighting multi-drug resistant bacteria. Phages thus have potential to be effective treatment option against almost every bacterial infection, but such possibility is yet under-explored. This study explores the possibility of using lytic phage therapeutically against 25 strains of drug resistant bacteria representing 12 genus. Kirby-Bauer methodology was employed for antibiotic sensitivity test and double-layer-agar assay was used for phage screening. Sewage water was used as phage source and plaques (clearing zones) were characterized visually. Multiple-host-range of isolated phages was analysed using spot-assay against all available bacteria of same genus. 34 phages were isolated from 5 sewage samples (mean=6.8/sample) against *E. coli* (16), *Klebsiella* (2), *Salmonella* Typhi/Paratyphi (13), *Shigella* (2), and *Citrobacter* (1). Except *Kelbsiella* phage, all other phages showed multiple-host-range. Most potent *E.coli* phage (TUEC3) lysed 53.33% of strains tested. Further, 80% of *E.coli* strains tested was lysed by at least one phage. The overall mean value of *E. coli* phage for multiple-host-range was 7 strains/phage. All *Salmonella* phage and *Citrobacter* phage showed lysis spots on 100% of strains tested. Heat killed phage lysate as control on spot-assay completely ruled out the possibility of false positive clearing zones by endolysins. Further, spectrophotometric analysis revealed that phages were able to kill both log and stationary phase bacteria effectively. Conclusively, phages can be used as an alternative to effectively cure multi-drug-resistant bacterial infections.

Genetic diversity of human isolates of *Blastocystis* in South India

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Blastocystis an atypical stramenopile, identified almost 100 years back but still many basic facts regarding its biology and pathogenic potential yet to be established, or are controversial. It is cosmopolitan in distribution, estimated to be found in more than 1 billion humans across the globe with verifying prevalence rate and known to parasitise mainly the large intestine of humans and a variety of animals. Due to its polymorphic nature identification by microscopy is obscure. Based on the ribosomal lineages different species of *Blastocystis* are designated as various subtypes (ST). Thus far 9 STs have been identified in humans. ST1 to ST4 together make up 90% of all human *Blastocystis* and ST3 & ST1 are the commonest subtypes found across the globe. On the other hand *Blastocystis* pathogenicity status in humans is debatable because the infection can be asymptomatic. However, molecular epidemiological data strongly suggests that certain subtypes such as ST3 & ST4 are more virulent and involved in intestinal/extra-intestinal manifestations. Further, inter and intra subtype variations may contribute to pathogenicity. Thus, molecular epidemiology is one of the important areas of *Blastocystis* research. Sequencing of 600bp of barcoding region of 18S small subunit ribosomal DNA is used to identify subtypes of *Blastocystis*. In India, data pertaining to *Blastocystis* are mainly based on microscopy, except a recently published molecular epidemiological survey on *Blastocystis* infection in healthy human population from Maharashtra, India. Thus, the aim of the present study is to explore the genetic diversity of *Blastocystis*. In this study the stool samples sent for routine parasitological examination were stored at -20ºC and later subjected for DNA extraction by using QIAamp DNA Stool Mini Kit - QIAGEN as per manufacturer instructions. Extracted DNA was quantified and subjected to *Blastocystis* specific PCR. Further, subtype analysis was done by sequencing the 600bp PCR product. Sequence results obtained from both the strands were assembled and subtype analysis was done using following database http://www.pubmlst.org/blastocystis/. A total of 68 *Blastocystis* PCR positive samples were subjected for sequencing and as of now we have received results of 9 samples. Preliminary results confirmed the presence of *Blastocystis* ST3 allele 34 in six samples and ST1 allele 4 in three samples. With the further sequencing results we would be able to reveal the most prevalent subtype present in south Indian population as well as its association with humans. The present study will represent the first investigation of human *Blastocystis* subtype distribution among south India.

Curcumin embedded polymeric microcapsules as effective anti-cancer drug

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There has been increasing trend of using chemotherapeutic drugs in cancer treatment all over the world. However, the application of various chemotherapeutic agents is not able to prevent its recurrence. On the other hand, curcumin, one of the major active ingredients of turmeric (*Curcuma longa*), which is a natural antioxidant, possesses multifunctional characteristics such as like anti-inflammatory and anti-cancer properties. Traditionally, curcumin has been used against common cold and stomach pain and has been found to enhance the immune system of the body. However, it has been demonstrated that the bioavailability of curcumin is a major concern. In this research, the curcumin 100µM was employed in sub-maxillary carcinoma cell (A-253) with the goal of increasing the bioavailability of this natural polyphenol based drug *via* micro-encapsulation. Further, the work has been aimed at enhancing the stability of the drug. Uniform sized hydrogel microcapsules were prepared using poly(lactic-*co*-glycolic acid) (PLGA) as a medium with the help of mono-nozzle ultrasonic atomizer allowing the curcumin particles to embed therein. Up to 30 days, curcumin drug was released from the microcapsules using 5% Tween solution as a releasing agent which showed an increment of the release rate up to 45%. Similarly, *in vivo* histological analysis showed that the curcumin drug along with natural hydrogel has significant suppressive action against (A-253) carcinoma cell line. The release of the drug from the microcapsules was found to be highly effective with reasonable tumour inhibition activity without any toxicity.

An interventional study on effectiveness of nutritional educational on knowledge, attitude and practice regarding anemia among students at Belgaum, Karnataka, India

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Anemia is one of the common public health problems mostly among young children, adolescent girls and pregnant women. According to World Health Organization, there are two billion anemic people worldwide. With the objectives to study the effectiveness of nutritional education on knowledge, attitude, and practice regarding anemia among school students, an interventional study was conducted among total of 115 students (57 students from standard V A and 58 students of standard VI A) of school chosen purposively of Belgaum district, India during January – October 2013. Pre-test was done prior to the intervention using pre-tested structured questionnaire and nutritional education on anemia was provided for three months as interventional package. Then, post-test was taken and data were analyzed using paired t-test. The paired t- test showed the significant increase in the knowledge, attitude and practice regarding anemia among the students in pre-post scores. The significant difference of 47.4% was found in knowledge of Anemia ($p<0.05$) and 20.7 % ($p< 0.05$) difference was found in positive attitude. Similarly, increase in frequency of consumption pattern of iron rich foods especially ragi, jaggery, green leafy vegetables and sprouted grains was observed. The package of nutritional education resulted in improvement of knowledge and change in attitude regarding anemia as well as increase in consumption of foods rich in Iron. Therefore, it can be concluded that nutritional education is the appropriate, cost-effective and sustainable method for lowering the cases of anemia.

Emergence of *Salmonella* Paratyphi A as the cause of enteric fever

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Enteric fever due to *Salmonella* Paratyphi A has become a threat across the world and its increasing antibiotic resistance is one of the major public health problems in developing nations. The aim of the study was to isolate enteric fever causing *Salmonella* species from blood samples, identify the serotypes and perform antibiotic susceptibility test to determine their prevalence and antibiotic resistance. A total of 837 blood samples were collected from the enteric fever suspected patients requested for blood culture visiting Helping Hands Community Hospital, Chabahil during the period of May 2014 to January 2015. Further processing of the samples was done in the laboratory of the same hospital where samples were inoculated in BHI broth and incubated at 37^0C for 24 hours and observed every 24 hours up to 7 days. If any visible changes took place, sub-culture was done in MacConkey and Blood agar. The isolated organisms were subjected to identification using biochemical tests. Disc diffusion method was used to find the antibiotic resistance pattern of the isolates. Out of 837, 44 (5.3%) samples were culture positive. Among them 25 (56.8%) were *Salmonella* Typhi and 19 (43.2%) were *Salmonella* Paratyphi A. Nalidixic acid resistance pattern was observed in 39 *Salmonella* isolates of which, resistance of *Salmonella* Typhi and *Salmonella* Paratyphi A were 92% and 84.2% respectively. Association was observed between resistance of the *Salmonella* isolates to nalidixic acid and ciprofloxacin (p=0.048). *Salmonella* Typhi showed 100% susceptibility towards chloramphenicol. *Salmonella* Paratyphi A was found to be more resistant towards conventional antibiotics chloramphenicol (5.3%) and cotrimoxazole (10.5%) compared to *Salmonella* Typhi for which the resistance were 0 and 4% respectively. Out of 19, 1 (5.3%) *Salmonella* Paratyphi A was multidrug resistant. Increasing prevalence and higher drug resistance in *Salmonella* Paratyphi A are potential signs of its disease inducing property.

In vitro clonal propagation and genetic fidelity of a medicinal orchid *Cymbidium aloifolium* (L.) Sw. by using RAPD-PCR technique

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Cymbidium aloifolium (L.) Sw., is one of the threatened epiphytic orchids of Nepal with high medicinal and ornamental values. The paste of leaves and roots were extensively used over dislocated bones and also in the treatment of burns, fever and chronic illness. Rapid agricultural development, uncontrolled deforestation, over exploitation and indiscriminate collection techniques decreases the population of this orchid species at an alarming rate from nature. Hence, the present study was conducted to preserve this multiutility orchid by establishing an efficient *in vitro* regeneration protocol using encapsulated protocorm called artificial seed. Protocorm was induced from seed explants on Murashige and Skoog (MS) medium and encapsulated using 2, 3 and 4 % sodium alginate solution and 0.2 M calcium chloride dihydrate ($CaCl_2.2H_2O$) solution. Different strengths of MS medium (full, half and quarter) and MS medium supplemented with 0.5 mg/l 6-benzyl aminopurine (BAP) and 0.5 mg/l α-naphthalene acetic acid (NAA) were tested for germination and subsequent development of artificial seeds. The maximum percentage of germination as well as shoot proliferation was observed on 3% artificial seed (7.16±1.77 shoots and 4.5±0.99 roots per culture) followed by 2% and 4% on full strength of MS medium. During plant tissue culture, the chances of somaclonal variation may occur. So, the clonal fidelity of *in vitro* regenerated plants was investigated using random amplified polymorphic deoxyribonucleic acid (RAPD) which detected 92% of genetic stability among the regenerants. The amplification bands of the regenerated plants showed similar banding patterns to that of the mother plant showing homogeneity of the tissue cultured plants. The plants were then acclimatized effectively with survival percentage 85% in a greenhouse using a rooting medium of crushed sterile cocopeat, brick, litter (1:1:1) topping with sphagnum moss. This study provides a basis for *ex-situ* germplasm conservation and clonal propagation of *C. aloifolium*.

Prevalence of intestinal parasitosis among school children in rural village of Chitwan district, Nepal

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Intestinal parasitosis is one of the major public health problems in Nepal, particularly among rural children.. We studied the prevalence of intestinal parasitic infections in school children of a village in Chitwan District of Central Nepal during a pre-monsoon season (July-August 2014). A total of 152 children (aged 4 to 18 years) who provided stool samples in a clean, dry and screw-capped plastic container were included in this study. School children consisted mainly of *Chepang* (indigenous nationality belonging to highly marginalized group), *Tamang* (indigenous nationality belonging to marginalized group) and *Gurung* (indigenous nationality belonging to disadvantaged group) ethnic groups. Stool samples fixed in 10% formal saline were examined by formal-ether sedimentation technique at Laboratory of Shi-Gan Intl' College of Science and Technology.Overall prevalence of parasitosis was 27.0% (41/152). Positive rate was significantly higher among *Chepang* ethnic group (36.5%) compared with others (14.9%) nationalities (p=0.003). Prevalence was marginally higher in girls (30.9%) and children aged ten and above 10 years (29.6%) than in boys (23.9%) and children below 10 years (22.3%). Significantly higher parasite positive rate was found among children drinking shallow-well water (42.2%, 24/57) than those drinking tap and stream water (p=0.001). Altogether, five types of parasites were found. Protozoa were most predominant over helminthes and *Trichiuris tichiura* was the only helminth parasite detected. This appeared to be due to seasonal distribution of anti-helminthic during recent years. Among protozoans, *Giardia lamblia* was most common, followed by *Entamoeba coli*, *Entamoeba histolytica* and *Endolimax nana*.

Cholera Outbreaks in Nepal

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Cholera is thought to be one of the oldest disease described in *Sanskrit* dating 5000 BC. It is an acute diarrhoeal disease caused by toxigenic *Vibrio cholerae*, and is still endemic in over four dozens of countries worldwide with large epidemics in some developing countries. Seventh pandemic cholera outbreak in Asia in 1961 had affected billions of people with over one million of death. In Nepal, cholera outbreak had been documented since 1823AD. Few cholera outbreaks were reported in 18th and 19th centuries in Nepal but many outbreaks after late 19th century. Most of these outbreaks occurred during rainy seasons and were associated mainly with *V. cholerae* O1 Ogawa strains. Our previous studies have also revealed the existence of *V. cholerae* O1 Ogawa in the sewage and water distribution system of Kathmandu Valley. However, a comprehensive geo-chronological review of cholera outbreak in Nepal is not available. Therefore, the present study systematically describes about the reported cholera outbreaks in Nepal. This study will be helpful for the policy makers to implement effective intervention programs in order to control the future outbreaks of cholera in Nepal.

Bacteriological analysis of enteric fever and its antibiotic susceptibility pattern in children attending Kathmandu Model Hospital

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Enteric fever is endemic in Nepal and constitutes a major cause of morbidity and mortality primarily affecting the children and young adults, caused by *Salmonella enterica* serovar Typhi (*S*. Typhi) or serovar Paratyphi (*S*. Paratyphi) A, B or C. A study was carried out at Kathmandu Model Hospital from March to September 2013 to determine the antibiotic susceptibility pattern of *Salmonella enteric*a serovars isolated from blood sample from the paediatric (≤ 15 years) patients suspected of enteric fever attending the Hospital. During this period, 300 blood samples were collected and inoculated into brain heart infusion broth and incubated aerobically at 37ºC for 18-24 hours. Macroscopic and microscopic observations and conventional biochemical tests were done to identify *S.* Typhi and *S*. Paratyphi. The isolated organisms were tested for antimicrobial susceptibility by using modified Kirby Bauer technique. Antimicrobial susceptibility test by disc diffusion and MIC values were analyzed using WHONET 5.6 software and statistical analysis was done by using Statistical Package for the Social Sciences version 17.0. Out of 35 positive cases, 30 isolates (85.72%) were *S.* Typhi and 5 isolates (14.28%) were *Salmonella* Paratyphi A. The highest incidence of infection was seen in age group 11-15 years (51.43%). All *Salmonella* spp. were sensitive to first line drugs (amoxycillin, chloramphenicol and cotrimoxazole), cephalosporins and azithromycin. Eighty percent isolates were resistant to nalidixic acid (NAL) while 20% ciprofloxacin (CIP) and 82.85% ofloxacin (OF) sensitive in disc diffusion test. The MIC value of 7 nalidixic acid sensitive (NAS) (16 µg/ml) isolates for CIP was ranged (0.25-0.5µg/ml) and for OF (0.25-1µg/ml) results both CIP MICs and NAL MICs within the susceptible range. The scatterplot correlating the MICs of CIP/OF and NAL illustrates the simultaneous presence of NAL sensitive and decreased CIP susceptibility. Rolled back of first line drugs be the drug of choice if high MIC value is observed for fluoroquinolones.

Serotyping and antibiotic susceptibility patterns of *Vibrio* and *Shigella* isolates from diarrheal patients visiting a tropical and infectious diseases hospital in Central Nepal

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Enteric and diarrheal diseases are the major infectious diseases in developing countries including Nepal. Poor water and sanitation play a role in outbreak of diarrheal disease. Lack of proper nutrition and antimicrobial resistance gained by microbes cause second leading cause of death of children below five years. Early diagnosis of disease and proper antibiotic treatment can significantly reduce the disease burden. The objective of this research was to understand the recent blueprint of antimicrobial resistance of major infectious agents (*Vibrio sps* and *Shigella sps*) to assure the proper antibiotic treatment. Stool samples of patient's visiting the hospital laboratory were processed following standard microbiological protocol and identified by biochemical and serological test recommended by the Clinical Laboratory Standard Institute. Antibiotic susceptibility test was performed by modified Kirby-Bauer disk diffusion technique. Among the 640 samples analyzed, 50 samples were found to be positive for enteric bacterial pathogens of which 29 were *Shigella* (4.5%) and 21 were Vibrio (3.2%), the total prevalence being 7.8%.The distribution of bacterial enteropathogens showed that *Vibrio cholerae* was found to be highest with (3.2%), followed by *Shigella flexneri* (2.6%), *Shigella sonnei* (1.2%), *Shigella dysenteriae* (0.5%), and *Shigella boydii* (0.2%). All *Vibrio cholerae* strains belonged to the serogroup O1 and serovar Ogawa. Among the *Shigella* spp, *Shigella flexneri* 17(59%) was the highest followed by *Shigella sonnei* 8(28%), *Shigella dysenteriae* 3(10%) and *Shigella boydii* 1(3%) respectively. All the *Vibrio* isolates were sensitive to cefotaxime while 71% were sensitive to tetracycline and 29% intermediately sensitive but 100% and 90.4% were resistance to co-trimoxazole and nalidixic acid respectively. *Shigella* isolates were mostly susceptible to cefotaxime (97%) while ciprofloxacin (48%) and ofloxacin (55%) were less effective antibiotics. These results on the prevalence of enteropathogens and their antibiotic resistance pattern may help to guide the accurate choice of therapy.

Study of Antioxidant Activity and Antimicrobial Property with Phytochemical Screening of Fruits of *Ficus* Plants Found in Nepal

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Ficus is a pantropical plant belonging to the moraceae family. Out of 36 species found in Nepal, 21 are known to have indigenous medicinal values while some are religiously important. Healthy, growing, adult stages fruits of thirteen species of *Ficus* were collected from different locations in Pokhara, Nepal during 2014 January to 2015 February. Thus collected fruits were cleaned, chopped into pieces and dried properly. Cold maceration was done with 100% methanol. The total phenol content of the extract was determined by folin-ciocalteu method and total flavonoid by $AlCl_3$ colorimetric technique. *In vitro* antioxidant activity was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. Antibacterial assay was carried out against *Escherichia coli* and *Staphylococcus aureus* by Kirby-Bauer disc diffusion method. Phytochemical screening was done by chemical methods. All species of *Ficus* under study showed anti-oxidant activities. Among them, extracts of *Ficus semicordata var.semicordata* and *Ficus auriculata* showed potent antioxidant activity with EC_{50} of 10.83 µg/ml while *Ficus lacor, Ficus rumphii* and *Ficus ariculata* showed antibacterial property against the gram positive bacteria, *Staphylococcus aureus*. Total polyphenol content of the extracts ranged from 34.375 to 110.375 µg GAE/mg and flavonoid content ranged from 21 to 335 µg QE/mg. Both the total phenolic and flavonoid content was high in extracts from *Ficus glaberrima*. Phytochemical screening of the *Ficus* fruits showed the presence of reducing sugar, phenolics, flavonoids and saponin. The results of this study shows that fruits of *Ficus* plants possesses anti-oxidant and antibacterial activities which can be further exploited for future medicinal uses.

Betulin attached to functionalized carbon nanotubes shows better efficacy against *Leishmania* parasite

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Leishmaniasis is classified as a major tropical disease by the WHO. Search for a successful vaccine against the parasite is still elusive. The main stream of treatment solely relies on chemotherapy. We report a novel anti-leishmanial formulation of betulin (BET) attached to functionalized carbon nanotubes (f-CNTs). We conjugated betulin, a pentacyclic triterpenoid secondary metabolite; to carboxylic acid chains on f-CNTs to obtain BET attached functionalized carbon nanotubes (f-CNT-Bet). The drug release profile demonstrated a fairly slow release of BET. The in-vitro cytotoxicities of BET, f-CNT and f-CNT-BET on J774A.1 macrophage cell line were

211.05±7.14µg/ml; 24.67±3.11µg/ml and 72.63±6.14µg/ml, respectively. The IC_{50} of BET and f-CNT-BET against intracellular *Leishmania donovani* amastigotes were 8.33±0.41µg/ml and 0.69±0.08µg/ml, respectively. The results demonstrate better anti-leishmanial efficiency of f-CNT-BET formulation than BET alone and with no significant cytotoxicity observed on host cells.

Fecal carriage of vancomycin-resistant enterococci in hospitalized patients and those living in the community in western Nepal

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Enterococcus spp have emerged as nosocomial pathogens over the last decade. The gastrointestinal colonization by multi drug resistant *Enterococcus* has been increasing worldwide. Hospitalized patients have an increased risk for infection or colonization by drug resistant *Enterococcus* compared to individuals living in the community. In Nepal, no systematic study is available regarding the prevalence rate of *Enterococcus* spp colonization. Therefore, the present study was initiated to determine the rate of fecal carriage of *Enterococcus* spp in hospitalized patients and in patients living in the community. Stool specimens or rectal swabs were collected from 141 community subjects and 129 hospitalized patients. All specimens were inoculated onto bile-esculin agar plates. Colonies growing on agar with a dark brown halo were identified by using conventional biochemical tests. Antibiotic susceptibility tests were performed using Kirby-Bauer disk diffusion method. The Minimum inhibitory concentration [MIC] for vancomycin was performed employing E-test strips (Hi-Media, Mumbai, India). Out of a total 270 study subjects 142 (52.59%) yielded *Enterococci*. Amongst these, 99 (69.71%) were from community subjects and 43(30.28%) were from hospitalized patients. Overall rate of colonization by MDR *Enterococci* was seen amongst 33.09% (47/142) subjects. Of these 47 MDR strains, 31 (72.09%) were isolated from 43 hospitalized patients, whereas only 16 (16.16%) were obtained from the 99 community subjects (χ^2=42.35; p<0.001). Vancomycin Resistant *Enterococci* (VRE) colonization rate was 6.33% (9/142); 18.6% (8/43) and 1.01% (1/99) respectively being from hospitalized patients and community subjects (χ^2=15.63; p<0.001). High rate of colonization by MDR *Enterococcus* and VRE in hospital in-patients is alarming. Further studies are warranted to elucidate the origin and the epidemiology of vancomycin resistance.

Bacteriological etiology and antibiogram of urinary tract infection isolates in pediatric patients

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Urinary tract infection (UTI) is the major health burden with high morbidity in paediatrics and demands especial attention due to its complications (e.g. renal scarring and end-stage renal disease). The study aimed to isolate and identify bacteriological uropathogens and determine their antibiogram profile. Ethical approval for this research was obtained from the Ethical Review Board, National Health Research Council (NHRC). A total of 353 samples, from children up to age 13 years, were investigated during the study period (July 2014 to January 2015). Antibiotics susceptibility test of isolates were performed by Kirby-Bauer disc diffusion method. 64 samples showed significant bacteriuria, with *E. coli* being significantly the predominant isolate (p<0.05). Gram negative bacteria (97%) were predominant compared to Gram positive bacteria (3%). The frequency of UTI was found significantly higher in early aged children, especially during first year of life (p<0.05). Among 64 positive samples, 36 samples showed bacteriuria along with pyuria and 28 samples showed bacteriuria without pyuria. Bacteriuria was found to be significantly associated with pyuria (p<0.05). Amikacin and nitrofurantoin antibiotics were found most effective among *E. coli* isolates. And, imipenem and amikacin were found most effective among other Gram negative isolates. The study showed high prevalence of antibiotics resistant isolates in paediatric UTI cases indicating need of routine surveillance of epidemiology and antibiogram profile to improve efficacy of empirical therapy.

Bacteriology of bloodstream infections in diabetic and non-diabetic patients under the course of hemodialysis

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Patients under the course of haemodialysis are usually immunocompromised and are more susceptible to blood stream infection. This infection is the most common cause of mortality in such patients. In general, infectious diseases are more frequent and serious in patients with diabetes mellitus, which potentially increases their morbidity or mortality. A cross-sectional study was conducted for a period of six months (June 2013 to December 2013) in National Kidney Center of Nepal to determine the prevalence of bacteremia in diabetic and non-diabetic patients undergoing hemodialysis course. Antimicrobial susceptibility pattern of the isolates was also determined. Blood samples were collected and inoculated in brain heart infusion biphasic media in blood culture bottle and incubated aerobically at 37ºC for 18-24 hours. Macroscopic and microscopic observations and conventional biochemical tests were done to identify the isolated organisms. The isolated organisms were tested for antimicrobial susceptibility by using modified Kirby Bauer technique. Data were analysed using Statistical Package for the Social Sciences version 17. In this study, 100 patients of age above 15 years to below 89 years old were included. Among them 12% were diabetic. No association was found between the bacteremia and diabetes (p value 0.35). Gram-positive bacteria were found predominant (88.5%). Among them 69.6% were *Staphylococcus aureus*. The prevalence of gram negative bacteria was only 11.5% and they were *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. During antibiotic susceptibility test pattern of the gram-positive isolates, oxacillin was found to be least effective and ciprofloxacin was found to be most effective. Out of total 16 *Staphylococcus aureus* isolates 37.5% were found to be methicillin resistant.

Prevalence of intestinal parasitic infection among prisoners in Kathmandu, Nepal

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Prisoners are among the high risk population for intestinal parasitic infection and other contagious diseases. We studied the prevalence of intestinal parasitism among prisoners of a jail in Kathmandu, Nepal in 2014. A total of 400 prisoners (M: 282 and F: 118) imprisoned for at least 6 months and still under imprisonment were included in this study. Morning stool samples were collected into a clean, dry and screw capped wide mouthed plastic container from each of the prisoners. Containers were distributed one day earlier with proper instruction on sample collection. Stool samples were transported to the research lab of Shi-Gan International College of Science and Technology and were fixed using 10% formal saline. Samples were examined employing formal ether sedimentation concentration technique. A total of 6% samples were positive for intestinal parasites; 2.5% of total sample were found positive for helminthic infection. *Trichuris trichiura* (1.5%), *Ascaris lumbricoides* (0.25%) and Hookworm (0.4%) were detected as helminthes and 3.5% of total samples were positive for protozoan parasites. Protozoan detected were *Giardia lamblia* (3.6%), *Entamoeba histolytica* (0.8%), *Entamoeba coli* (0.8%) and co-infections of *E. coli* and *E. nana* (0.4%) and *E. coli* and *G. lamblia* (0.4%) were found. The prevalence of intestinal parasitism was found higher in female(7.6%) compared to that in male(5.3%) and the same, according to seasonal variation, showed no significant difference; 6.03% before the starting of rainy season ,5.9% at the end of rainy season. Despite of proper health education inside prison, knowledge about importance of sanitation, administration of anti-parasitic drugs to all prisoners at the interval of 6 months, the incidence of the parasitic infection may be due to congestion of population and the lack of implementation of the proper sanitary practice. Based on these findings, continuation of administration of anti-parasitic drugs and strict implementation of sanitary practice in all prison settings is recommended.

Serological and entomological study of Dengue in Dang and Chitwan districts

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Dengue is the mosquito-borne viral disease in the world which is transmitted by mosquito of genus *Aedes*. It is important to diagnose dengue fever (DF) and its vector for patient management and control of dengue outbreaks. In this study, DF were detected by IgM capture Enzyme Linked Immuno-Sorbent Assay (ELISA). A total 264 serum samples were collected from Chitwan and Dang district from June to November of 2013. The anti-dengue IgM positivity was found to be 19.3% (n=51), of which 22.4% were female and 16.2% were male. The highest positive cases were from the age group 15- 50 which was 19.6%. Clinical features of seropositive cases were anorexia (OR=7.6), nausea (OR=2.9), headache (OR=2.2), retro- orbital pain (OR=2), skin rash (OR=2) and myalgia (OR=1.3) which showed that patient with such symptoms were more likely to develop DF. Hematological features like thrombocytopenia and leucopenia were found significantly associated with the DF. Discarded tires were found as the common breeding habitats of the dengue vector. Higher the vector index higher was the seropositivity. The pH and salinity of water of breeding habitats were found between 6.9 to 8 and 0.2 ± 0.1ppt to 25ppt respectively. This study could be helpful for the health authorities and public health workers for early diagnosis of DF and for the preventive measures to be adopted in the epidemic and possible epidemic areas.

Prevalence of foodborne pathogens and spoilage microorganisms and their drug resistant status in different street foods of Dhaka city

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This study was designed to evaluate the microbial status of street foods. For this assessment, thirty nine street foods samples of 13 kinds were collected from Motijheel area of Dhaka city. These samples were analyzed for total bacteriological quality, different foodborne pathogens and spoilage microorganisms. The average aerobic bacterial and coliform count varied from 3.0 ± 0.04 log CFU/g to 8.8 ± 0.02 log CFU/g and 2.0 ± 0.01 log CFU/g to 7.5 ± 0.02 log CFU/g respectively. Presence of *Salmonella* spp. and *Escherichia coli* (O157, O111, O26) in boiled motor and cucumber samples and other *E. coli* in pitha, jilapi, tehari, amra and cucumber samples, coliform bacteria in 28 samples, *Enterococcus* spp. in ten samples, *Listeria* spp. in fifteen samples, *Yersinia* spp. in ten samples, *Enterobacter sakazakii* in eight samples, and *Staphylococcus* spp. in 39 samples was observed. The presence of spoilage organisms *Bacillus* spp. in twelve samples, *Pseudomonas* spp. in fifteen samples and lactic acid fermenting bacteria (LAB) in 24 samples was observed. The isolated micoorganisms were then tested for antibiotic sensitivity and the results revealed that all *Salmonella* spp., *Escherichia coli* O157, O111, O26 and others *E. coli, Enterobacter* were multi drug resistance and same was true for all the coliform, *Listeria* spp., *Pseudomonas* spp., and LAB. *Staphylococcus* spp., *Bacillus* spp., isolates were resistant to many and some isolates were resistant to all the antibiotics tested. Reported plate count data represent the mean values of duplicated samples. Data were subjected to analysis of variance using the Microsoft Excel program (Redmond, Washington DC, USA). Significant differences in data were established by the least-significant difference at the 5% level of significance. This study demonstrated that, street foods of Dhaka City constitute a potential microbial hazard to human health.

Intestinal parasites among school going children of Kathmandu Valley, Nepal

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Intestinal parasites are still a major causative agent of death in developing country like Nepal. Despite the presence of enormous economical accessible medication and treatment, million children are still suffering from these diseases. The objective of this study was to find out the enteropathogens in school going children along with other variables. The study was conducted at Public Health Research Laboratory and samples were collected from ten different schools of Kathmandu valley from November 2014 to February 2015. A total of 455 samples were collected, with or without having the symptoms of diarrhea; were transported to the laboratory by maintaining cold chain and processed according to the standard guideline. The overall prevalence of intestinal parasites was 67 (14.72%), among them highest percentage were seen in female (16%). The highest number of protozoa (12.3%) was seen compared to helminths (2.4%). The formal–ether concentration technique yielded 67 (14.72%) whilst 61 (13.4%) in direct microscopic technique. Among total, 59 (12.9%) single and 8 (1.7%) were mixed infections (both protozoa and helminth; protozoa and protozoa). The commonest intestinal parasite found was *Giardia lamblia* (59.7%) followed by *Entamoeba histolytica* (11.9%), *Cyclospora cayentensis* (8.9%), *Hymenolepsis nana* (5.9%), *Blastocystis hominis, Trichuris trichiura* and *Entamoeba coli* holding same (2.9%%) on the other hand *Ascaris lumbricoides, Enterobious Vermicularis* and *Strongyloides stercoralis* holding same (1.4%). The distribution of enteroparasites was found to be highest in age group 6-10 yrs (12.5%) in accordance in filter water user i.e. 10.9% (50/455) and in vegetarians i.e. 13.4% (61/455). After treatment with antiparasitic drugs to the positive child, on follow up after two months 5.9% (4/67) cases were found still positive. The study reveals the highest intestinal parasites was seen in school going children and concentration techniques is more effective; as well as required to check the efficacy of the existing antiparasitic drugs.

Prevalence of metallo-β-lactamase producing Gram Negative Bacteria isolated from different clinical Samples in a tertiary care hospital

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Metallo-β-lactamase production among gram-negative bacterial species such as *Serratia marcescens, Pseudomonas aeruginosa, Acinetobacter baumanii* and *Escherichia coli* have been recently reported with increasing frequency worldwide. The present study aimed to determine the prevalence of Metallo-β-lactamase producing gram negative bacterial isolates from a tertiary care hospital in Nepal. A total of 362 gram negative isolates recovered at the microbiology laboratory of Nepal Medical College and Teaching Hospital, Kathmandu during the six months period (May to November 2014) were included in the study. Antibiotic susceptibility test of the isolates were performed by Kirby- Bauer disc diffusion method and Metallo β- lactamase (MBL) production, detected by combined disk test using Imipenem with Ethylenediaminetetraacetic acid (EDTA). Among the gram-negative isolates, 5.80% (21/362) were found to be MBL positive with *Acinetobacter calcoaceticus baumanii* complex showing the highest prevalence i.e. 85.71% (18/21), followed by *P. aeruginosa* i.e. 14.29% (3/21). None of the other gram negative bacteria isolated from different samples (urine, pus, sputum and body fluid), analyzed for the study were found to be positive for MBL production during the study. Out of the 21 MBL positive isolates detected, 16 (76.19%) were from hospital in-patient and remaining 5 (23.81%) from hospital out patients, the finding was statistically insignificant ($p>0.05$). Meanwhile, the finding of highest prevalence of MBL positive isolates from urine and pus sample i.e., 7 (33.33%) for both and lowest prevalence from body fluid i.e., 3 (14.29%) was statistically insignificant ($p>0.05$). Polymixin B i.e. 24 (100%), followed by Imipenem i.e. 22 (91.67%) were found to be the most sensitive drug among the *Pseudomonas* isolates likewise, Tigecycline i.e. 30 (85.71%), followed by Imipenem i.e. 24 (68.57%) were found to be sensitive among *Acinetobacter* spp. Moderately higher prevalence of MBL-producing gram negative bacteria was observed during the study, warranting a national surveillance for routine monitoring of MBL producing bacterial isolates.

Prevalence of HCV and HBV co-infection among clinically diagnosed HIV patients by using Nested-PCR

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The Common mode of transmission through cutaneous routes of several infectious diseases can result in Co-infection of such diseases among individuals. In the study of Nepal Health Research Council Hepatitis B virus (3.8%) and Hepatitis C virus (10%) blood borne viruses are among the most endemic infectious agents causing acute and chronic morbidity in Nepal. Pathogenesis of HBV and HCV relies on the immunity of the patient. Furthermore, person with HIV have decreased CD4 cells because of which they are prone to chronic hepatitis HBV and HCV. The project studied the prevalence of co infection of HCV and HBV among HIV patients. A total of 20 blood samples were collected from HIV patients visiting Bir hospital, Kathmandu, Nepal after informed consent. DNA was extracted from the blood sample for the detection of HBV whereas RNA extraction was done for identification of HCV sample. Nested PCR was performed using Kits (Genekam Ar Biotechnology) for specific amplification and finally agarose gel electrophoresis was carried out for visualization, analysis and interpretation of the results. Among total samples, 30% were found to be co infected with HCV and 45% of HBV. The co prevalence of male was found to be higher in HCV (66%) with the age groups of 46- 55 years whereas HBV (37%) to be higher in age group 30-49 years in case of female. The co-prevalence rates of HBV and HCV are significantly higher in the HIV patients which clearly indicate the need for HIV control programmers and vaccination to be promoted through public awareness as preventive strategy.

Prevalence and current antibiogram trend of bacterial isolates of urinary tract infections in outpatients at Helping Hand Community Hospital, Kathmandu

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Urinary Tract Infections (UTI) are the most common type of infection in both hospitalized and outpatients. We have determined the bacterial etiology of UTI, their frequency and antibiotic susceptibility pattern. Midstream 'clean catch' urine samples were aseptically collected from the patients visiting at Helping Hand Community Hospital,

Kathmandu. The samples were cultured on Cysteine Lactose Electrolyte Deficient (CLED) agar and incubated at 37°C for 18-24 hours. The positive culture were identified by colony characteristics, gram staining and biochemical tests using standard bacteriological methods and further processed for standard antibiotic susceptibility tests by Kirby-Bauer method. Out of total 152 urine samples, 59 (38.81%) gave significant bacterial growth. The majority of bacteria were gram negative 52 (88.14%). The prevalence of bacterial isolates was higher among females (48.84%) than males (25.76%), ($p<0.001$%). The most common isolated bacteria was *Escherichia coli* 35 (59.32%), followed by Coagulase Negative Staphylococcus (CONS) 5 (8.47%), *Salmonella enterica* 4 (6.78%), *Proteus mirabilis* 2 (3.39%), *Proteus vulgaris* 2 (3.39%), *Klebsiella pneumoniae* 2 (3.39%), *Salmonella typhi* 2 (3.39%), *Pseudomonas* spp 2 (3.39%), *Staphylococcus aureus* 2 (3.39%), *Citrobacter spp* 2 (3.39%), *Acinetobacter baumannii* 1 (1.69%),

Acinetobacter spp 1 (1.69%), *Proteus vulgaris* 1 (1.69%). The frequency of bacterial isolates was in range from 8 to 72 years with most values at the low end. The median age of bacterial isolates for women is 30 year, and for men is 59 year. The most predominant organism, *Escherichia coli,* was found to be highly sensitive to Nitrofurantoin (97.41%) followed by Gentamicin (88.57%), Norfloxacin (80%) and was resistance to Nalidixic acid (71.42%) and Amoxycillin (80%). Gram positive bacterial isolates were 100% sensitive to Gentamicin, Nitrofurantoin. Other gram negative bacterial isolates were mostly sensitive to Gentamicin. A majority of bacteria were resistant to commonly used antibiotics.

Light Emitting Diode (LED) fluorescent microscopy: An alternative to screen tuberculosis in Nepal

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Screening of tuberculosis (TB) patients and laboratory diagnosis of causative agent plays a significant role to control and extirpate the disease, because, in the developing countries including Nepal, rapid and accurate diagnosis of active TB is still a far cry due to the lack of adequate tools and techniques which directly affect the global control of the disease. In Nepal, 45% of total population is infected with TB of which 60% are in the productive age group (15 to 45 years). There is an urgent need for an appropriate TB diagnostic tool that is simple, rapid, sensitive and specific and can be made widely available so that 3 million missing TB cases (WHO) and new cases as well as relapse cases can be detected. This study was performed in German Nepal Tuberculosis Project (GENETUP) laboratory with an objective to evaluate the diagnostic efficacy of two staining techniques (Ziehl-Neelsen and LED fluorochrome staining) for the detection of acid fast bacilli (AFB) in comparison to culture. A total of 325 samples were processed in this study in which 219 were from male and 106 were from female, among them, 56% of the samples were from the productive age group (15 to 45 years). From the total samples processed, 97 were AFB positive by ZN microscopy and 104 by LED microscopy. Culture showed 121 positive results while 7 cultures were contaminated. Considering culture as gold standard, sensitivity of ZN and LED microscopy were found to be 70.8% and 72.5% and specificity were 94.9% and 92.4% respectively. While working, LED microscopy technique was found to be very easier and faster than the conventional technique (ZN microscopy). From this study, it is concluded that LED microscopy is potentially more suitable for laboratories to screen TB in resource-limited settings and countries like Nepal where the TB burden is comparatively higher so that it can be used as an alternative to ZN microscopy for TB detection.

Characterization of Structural and Antibacterial Properties of some Heavy Metals Containing Ayurvedic Drugs

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Ayurveda (literally Science of Life) uses integrated treatment system to cure diseases and promote human health, in which herbal formulation is an important part. The use of Ayurvedic formulations was the sole medicinal practice in South Asia before the introduction of modern medicines. Besides plant formulation, other inorganic components are also added to reinforce the drug's effect. Among those inorganic components, heavy metals form an important part. The use of metals in drug manufacture is termed as Rasasastra. On the other hand, heavy metals, frequently used in Ayurvedic medicines, are supposed in general to be poisonous as well as carcinogenic. However, these days many experiments are being performed using these compounds for the treatment of disease especially chronic ones including cancer. We can assume that these metals have toxic effects on microorganism as well. In this work, in an attempt to understand the structure and anti-bacterial properties of some heavy metals containing Ayurvedic drugs, we have performed spectroscopic, microscopic and antibacterial assay. The drugs were received from the government controlled Ayurvedic manufacturer Singhadurbar Vaidyakhana Vikas Samiti (SDVKVS), Kathmandu, Nepal. The drugs investigated include Arsenic containing (such as Rasamanikya and Swashkuthar Rasa), lead containing (such as Mahayogaraja Guggulu) and mercury containing (such as Rasasindur, Sutasekhar Rasa, Siddhapraneshvara Rasa and Navarasa) formulations. It was found that some of the formulations were found to be strongly sensitive against Streptococcus and Escherichia coli etc.

Prevalence of Intestinal Parasitosis among School Children of Phedikhola, Syanja, Nepal

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Intestinal parasitosis is a serious public health problem in developing countries like Nepal. It is more common in children and causes significant morbidity including mortality.A cross-sectional study was done to find the prevalence of intestinal parasitosis among school children of Phedikhola VDC in Syangja district. A total 372 fecal sample collected from school children studying at Siddhartha Higher Secondary School (n=209) and Little Flower English Boarding School (n=163) were included. About 5 gm of fresh stool was collected in clean, dry and screw capped plastic container from each student and was examined macroscopically for the color, consistency, presence of blood, mucus and adult worm or worm segments. Then, mixed with equal volume of 10% formal saline and transported to laboratory of Shi-Gan International College of Science and Technology at Maharajgunj, Kathmandu. The microscopic examination of formalin fixed specimen was done by formal ether sedimentation technique. Out of 372 samples, 72(19.3%) samples were positive of intestinal parasitosis. The prevalence rate in boys were 52.8%(38/72) and girls were 47.2% (34/72). Of the positives *Giardia lamblia* (47.2%) was most common followed by *Entameoba coli* (26.4%), *Endolimax nana* (20.8%) and *E. histolytica* (13.9%). No helminth was detected. The percentage of single infection was higher (90.3%) than multiple infection (9.7%). The higher rate of infection was found in the children aged 11-16 years.The prevalence of parasitic infection with age-group was found to be statistically insignificant (p=0.79). The low incidence of parasites may be due to administration of anti-helminthic drugs to the children at the interval of every six months. However, proper sanitation, pure drinking water and public awareness must be continued.

Key Words: Intestinal parasitosis, school children, Phedikhola, Syangja

Characterization of a novel Topoisomerase IA of *Leishmania* to develop it into a potent anti-leishmanial drug target

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Leishmaniasis is predominantly a dreadful neglected tropical disease caused by *Leishmania* species and transmitted into humans through sandflies. Lack of vaccines and proper vector control measures leaves chemotherapy as the only choice, which unfortunately is plagued due to chemoresistance (1). Hence there is an ardent need of novel drug targets. Topoisomerases are essential enzymes involved in vital cellular processes and drugs targeting it have long been used for cancer chemotherapy (2). On BLAST search in the *Leishmania* Genome database, we identified LdBPK_210180.1, a unique DNA Topoisomerase IA (LdTopIA). Next multiple sequence alignment using CLUSTALW shows that LdTopIA is homologous to *E .coli* and *M.smegmatis* TopoI and bears a similar active site motif. Using the crystal structure of *E.coli*TopoI (PDB ID-1CY1) we generated a homology-modeled structure of LdTopIA and used Raswin to highlight the conserved active site pocket. Further on, LdTopIA could functionally complement the *E.coli* strain RFM475 (TopoI null-DNA gyrasets mutant) (3). Analysis of DNA binding and in vitro plasmid relaxation using LdTopIA would help its further characterization. LdTopIA similar to *E.coli*TopoI may be required to resolve transcriptional R-loops which otherwise would induce transcriptional-block and cell death (4).
The absence of TopoIA in humans makes LdTopIA a novel anti-leishmanial target, which can be inhibited by the potent *E.coli*TopoI inhibitor haloemodin (5). Further modifications of haloemodin would be carried out to increases its potency, hydrophilicity, cell permeability, bioavailability etc. These dendrimers can be loaded onto surface functionalized liposomes and specifically targeted to *Leishmania* infected cells so as to inhibit LdTopIA and thereby eliminate the parasite.

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2nd International Conference on Infectious Diseases and Nanomedicine – 2015 (ICIDN-2015)
December 15-18, 2015, Kathmandu, Nepal

ICIDN 2015 - CONFERENCE TIME TABLE

DAY I. TEUSDAY, DECEMBER 15, 2015

Pre-conference Workshop: ***Current Issues and Challenges in Infectious Diseases & Nanomedicine Research***		
08:00 - 08:30		**Arrival of Participants; Welcome and Introduction**
		TUTORIAL LECTURES
08:30 - 09:10	TL1	*Shiba Kumar Rai, Nepal Medical College, Kathmandu University, NEPAL* **Research Challenges for Vector-Borne Diseases Elimination in Nepal**
09:10 - 09:50	TL2	*Prof Ananda M Chakrabarty (USA)*
09:50 - 10:20		**Tea/Coffee Break**
10:20 - 11:00	TL3	*Bal Mukunda Regmi, Institute of Medicine, Tribbhuvan University, Kathmandu, NEPAL* **Infectious diseases: Surveillance and control strategies**
11:00 - 11:40	TL4	*Rameshwar Adhikari, Central Department of Chemistry, Tribhuvan University, Kathmandu, NEPAL* **Nanosceince and Nanotechnology: What is it and What we can do?**
11:40 - 13:00		**Lunch Break**
13:00 - 13:40	TL5	*Abdul Haque, University Medical and Dental College, The University of Faisalabad, PAKISTAN* **Dengue Fever: Diagnostics and Therapeutics**
13:40 - 14:00		**Closing of the Workshop; Tea/Coffee**
16:30 - 17:00		**INAGURATION CEREMONY**
17:00 - 17:30	**OL1**	**Infectious Disease and Cancer: Drug Development with Common Efficacy** *Ananda Mohan Chakrabarty, University of Illinois College of Medicine at Chicago, USA*
17:30 - 18:00	**OL2**	**Carbon Nanotube and Its Medical Applications** *Mushahid Husian, M. J. P. Rohilkhand University, Bareilly (UP), India*
18:00 - 20:00		**Welcome Reception/Dinner & Social Programs**

NOTE: TL= Tutorial Lecture, OL= Opening Lecture, SL= Special Lecture

ICIDN 2015 - CONFERENCE TIME TABLE

DAY II. WEDNESDAY, DECEMBER 16, 2015

Keynote Lectures		
08:30 - 09:00	KL1	**How does Vaccinia Virus Interfere with Interferon? New Findings with an Old Vaccine** *Geoffrey L. Smith, University of Cambridge, Cambridge, UK*
09:00 - 09:30	KL2	**Development of Cancer Sensing Nanodevices using Metamaterial Nanostructures** *Mahi R Singh, The University of Western Ontario, London, Ontario, Canada*
09:30 - 10:00		**Tea/Coffee Break**
Symposium: Cellular and Molecular Microbiology of Infectious Diseases (I)		
10:00 - 10:20	IL1	**Infectious Diseases in Nepal: Status of Toxoplasmosis** *Shiba Kumar Rai, Nepal Medical College, Kathmandu University, Kathmandu, Nepal*
10:20 - 10:40	IL2	**Typhoid Fever Vs Typhus Fever in Nepal** *Buddha Basnyat, Patan Academy of Health Sciences, Kathmandu, Nepal*
10:40 – 10:55	OP1	**Molecular Studies on the Haitian variant *ctxB* gene and cholera toxin production in *Vibrio cholerae* O1outbreak strains isolated from India** *Lekshmi.N, Sabu Thomas* *Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India*
10:55 - 11:10	OP2	**Characterization of *Staphylococcus aureus* strains from skin and wound infection cases in Haripur and Abbotabad cities of Pakistan** *Muhammad Ali Syed, Sheer Ali, Maria Gul, Humaira Jamil, Maria Rukan, Malik Sarfaraz Ahmed, S. Habib Ali Bokhari, Zobia Noureen, Allah Nawaz Khan* *University of Haripur, Haripur, Pakistan*
11:10 - 11:25	OP3	**Human Papilloma Virus among Women Visiting a Health Facility in Kathmandu, Nepal** *Deepak Sharma Paudel, Bishnu Joshi, Ganesh Rai, Sunil Lekhak, Neetu Singh, Basant Pant, Shiba K Rai* *Shi-Gan International College of Science and Technology, Kathmandu, Nepal*
11:25 - 11:40	OP4	**Community and hospital acquired MRSA: Association of Panton Valentine Leukocidin genes** *Dharm R. Bhatta, Lina M. Cavaco, Gopal Nath, Kush Kumar, Abhishek Gaur, Shishir Gokhale, Dwij R. Bhatta* *Manipal College of Medical Sciences, Pokhara, Nepal*
11:40 - 12:00		***Group Photo: Please gather at the front part of the Congress Center***
12:00 - 13:00		**Lunch Break**
Symposium: Nanomaterials and Bio-medical Materials (I)		
13:00 - 13:20	IL3	**Nanoformulations in neurological disorders** *Bikash Medhi* *Postgraduate Institute of Medical Education & Research, Chandigarh, India*
13:20 - 13:40	IL4	**Morphology of Nanofibrous Materials for Medicinal Applications** *Jakub Sirc* *Institute of Macromolecular Chemistry, Prague, Czech Republic*
13:40 - 13:55	OP5	**Heart and liver regeneration in zebra fish using silver synthesis particle from *Marine Plant- In vivo*** *M. Syed Ali, V. Anuradha , N.Yogananth, Ms.Sathya* *Mohamed Sathak college of Arts and Science Sholinganallur, Chennai, India*
14:00 – 14:15	OP6	**Synthesis, Spectroscopic Characterization and Insulin Mimetic Activity of Oxovanadium(IV) Macrocyclic Complexes** *M. L. Sharma, S. K. Sengupta, O. P. Pandey* *Tribhuvan University, Tri-Chandra Multiple Campus, Kathmandu, Nepal*

14:15 - 14:30	OP7	**ZnO Nanoparticles and its Medical Applications in Cancerous Cell** *Renu Choithrani, Barkatullah University, Bhopal, India*
14:30 - 14:45	OP8	**Nanomaterials based electrochemical biosensors for medical applications** *Bal Ram Adhikari, Maduraiveeran Govindhan, Aicheng Chen* *Lakehead University, Thunder Bay, Ontario, Canada*
14:45 - 15:00	OP9	**Trick malaria parasites using nanomimics of host red blood cell membranes ?** *Adrian Najer, Cornelia G. Palivan, Hans-Peter Beck, Wolfgang Meier* *University of Basel, Basel, Switzerland*
15:00 - 15:30		**Tea/Coffee Break**
Symposium: Antimicrobials, Vaccines and Alternatives (I)		
15:30 - 15:50	IL5	**Competitive exclusion of foodborne pathogens by stimulating growth and production of bioactive components of *Lactobacillus casei*** *Debabrata Biswas, University of Maryland, Maryland, USA*
15:50 - 16:05	OP10	**Antibacterial and cytotoxic evaluation of different extracts of *Parthenium hysterophorus*** Sidra Shoaib, Muhammad Adil Rasheed, Muhammad Ashraf, Aftab Ahmad Anjum *University of Veterinary and Animal Science, Lahore, Pakistan*
16:05 - 16:20	OP11	**Epitope-based peptide vaccine design and target site depiction against Ebola viruses** *Md. Arif Khan, Mohammad Uzzal Hossain, S.M. Rakib-Uz-Zaman, Mohammad Neaz Morshed* *Military Institute of Science and Technology, Dhaka, Bangladesh*
16:20 - 16:40	IL6	**Treatment Options in a Post- Antibiotic Era** R. R. Bragg, van der Westhuizen, W. Coetzee, M., Lee, J-Y., Jansen, A.C., Theron, C., Boucher, C.E. *University of the Free State, Bloemfontein, South Africa*
16:40 - 18:40		**POSTER SESSION**

DAY III. THURSDAY, DECEMBER 17, 2015

Keynote Lectures		
08:30 - 09:00	KL3	**Significance of Vi negative isolates of *Salmonella enterica* serovar Typhi in causing typhoid fever** *Abdul Haque, University Medical and Dental College, The University of Faisalabad, Pakistan*
09:00 - 09:30	KL4	**Role of Molecular Tests in Laboratory Diagnosis of Syphilis** *Muhammad G. Morshed, University of British Columbia, Vancouver, BC, Canada*
09:30 - 10:00		**Tea/Coffee Break**
Symposium: Epidemiology and Infectious Diseases Surveillance Hall A (Parallel Session)		
10:00 - 10:20	IL7	**Human Rabies in Nepal: a-14-year experience from a tertiary central referral tropical infectious disease hospital** *Sher bahadur Pun* *Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal*
10:20 - 10:35	OP12	**Prevalence of extended spectrum beta-lactamases among uropathogenes at NU Hospital Bangalore** *V. Manjunath, S M Hegde, Solanki Mukherjee* *Microbiology Department, NU Hospital Bangalore, India*
10:35 - 10:50	OP13	***Salmonella enterica* serovar Typhi in Carcinoma Thyriod: A Case report** *Sumathi Gurusidappa, Jayshree Rudrapatna* *Kidwai Memorial Institute of Oncology, Bengaluru, India*
10:50 - 11:05	OP14	***Candida parapsilosis* onychomycosis, an unusual presentation in a child** HS Supram, Deependra Hamal, N Nayak, S Gokhale *Department of Microbiology, Manipal College of Medical Sciences, Pokhara, Nepal*
11:10 - 11:25	OP15	**Microsporidial keratitis in immunocompetent patients from North India** *Sonu Kumari Agrwal, Sumeeta Khurana, Kriti Megha, R Sehgal, Amit Gupta* *Department of Medical Parasitology, Postgraduate Institute of Medical Education and Research, Chandigarh, India*
Symposium: Cellular and Molecular Microbiology of Infectious Diseases (II) Hall B (Parallel Session)		
10:00 - 10:20	IL8	**Molecular characterization of acquired quinolone resistance in clinical isolates of *Salmonella* Typhi** *Punit Kaur* *All India Institute of Medical Sciences, New Delhi, India*
10:20 - 10:35	OP16	**Conventional Methods Of Methicillin Resistant Staphylococcus Aureus (MRSA) Detection** Surendra Kr. Madhup, Mukesh Neupane *Department of Microbiology, Dhulikhel Hospital, Kathmandu University Hospital, Dhulikhel, Nepal*
10:35 - 10:45	OP17	**Evaluation of Multiplex PCR using *MPB64* and *IS6110* primers for Rapid Diagnosis of Tuberculous Meningitis** *Sunil Prasad Lekhak, Laxmi sharma, Reema Rajbhandari, Pravesh Rajbhandari, Resha Shrestha, Basant Pant* *Annapurna Neurological Institute and Allied Sciences, Maitighar, Kathmandu, Nepal*
10:45 – 11:00	OP18	**A Molecular-Beacon-Based asymmetric PCR assay for easy visualization of amplicons in the diagnosis of trichomoniasis using pyruvate:ferredoxinoxidoreductase proprotein gene as target** *Subash C Sonkar, Daman Saluja* *Dr. B. R. Ambedkar Center for Biomedical Research, Delhi, India*
11:00 - 11:15	OP19	**Next generation sequencing to divulge genomic framework of *Helicobacter pylori*** *Binit Lamichhane, Mary Webberly, Eng Guan Chua, Fanny Peters, Alfred Chin Yen Tay* *University of Western Australia, Perth, Australia*
11:15 – 11:30	OP20	**Gene expression analysis of *Plasmodium falciparum* Dd2 strain using Whole Transcriptome Sequencing** *Hiasindh Ashmi Antony, Vrushali Pathak, Kanjaksha Ghosh, Subhash Chandra Parija* Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry, India
11:30 – 12:00	IL9	***Basanta Pant*** *Annapurna Neurological Institute and Allied Sciences, Maitighar, Kathmandu, Nepal*

12:00 - 13:00		**Lunch Break**
Symposium: Antimicrobials, Vaccines and Alternatives (II)		
13:00 - 13:20	IL10	**Antifungal drug resistance** *Niranjan Nayak* *Manipal College of Medical Sciences, Kathmandu University, Pokhara, Nepal*
13:20 - 13:35	OP21	**Detection of rpoB gene mutation in *Mycobacterium tuberculosis* by Amplification Refractory Mutation System-Polymerase Chain Reaction** *Hemanta Kumari Chaudhary, Mitesh Shrestha, Bal Hari Poudel* *Central Department of Biotechnology, Tribhuvan University, Kathmandu, Nepal*
13:35 - 13:50	OP22	**Identification and screening of novel antiretrovirals targeting HIV maturation** *Uddhav Timilsina, Bivek Timalsina, Ritu Gaur* *South Asian University, New Delhi, India*
13:50 - 14:05	OP23	**High-resolution melt (HRM) analysis for rapid detection of *Mycobacterium leprae* drug resistance mutations from leprosy patients from India** *Mallika Lavania, Astha Nigam, Ravindra Turankar, Itu Singh, Utpal Sengupta* *Stanley Browne Laboratory, The Leprosy Mission Community Hospital, New Delhi, India*
14:05 - 14:25	IL11	**Demonstration of the unbearable lightness of phage therapy targeting resistant bacteria** *Ronen Hazan* *Hebrew University, Jerusalem, Israel*
14:30 - 15:00		**Tea/Coffee Break**

Symposium: Drug Design, Drug Delivery & Tissue Engineering		
15:00 - 15:20	IL12	**Intelligent polymeric micro/nanoparticles with entrapped active agents** *Nirmala Devi, Tarun K Maji, Dilip K Kakati* *Gauhati University, Assam, India*
15:20 - 15:40	OP24	**Studies on degradable polyester based composites for biomedical application** *Jyoti Giri, Rameshwar Adhikari, Ralf Lach, Hai Hong Le, Hans-Joachim Radusch, olfgang Grellmann* *Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal*
15:40 - 15:55	OP25	**Synthesis of magnetic starch-iron oxide nanocomposite for controlled drug delivery** *Gunjan Bisht Thapa* *Kathmandu University, Dhulikhel, Nepal*
15:55 - 16:10	OP26	**Biodegradability and antimicrobial properties of polyvinyl alcohol (PVA) based chitosan composites** *Shanta Pokhrel, S. Lamichhane, K. D. Manandhar, P. N. Yadav, Rameshwar Adhikari* *Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal*
16:10 - 16:25	OP27	**Myxobacterial cytochromes P450 based whole cell biocatalyst: Generation of the novel drug derivatives from the tricyclic antidepressants, antipsychotics, antineoplastic agents and steroids** *Yogan Khatri, Martin Litzenburger, Fredy Kern, Rita Bernhardt* *Saarland University, Saarbruecken, Germany*
16:30 – 17:00		**Tea/Coffee Break**
17:00 – 18:00		**Farewell & Valediction**

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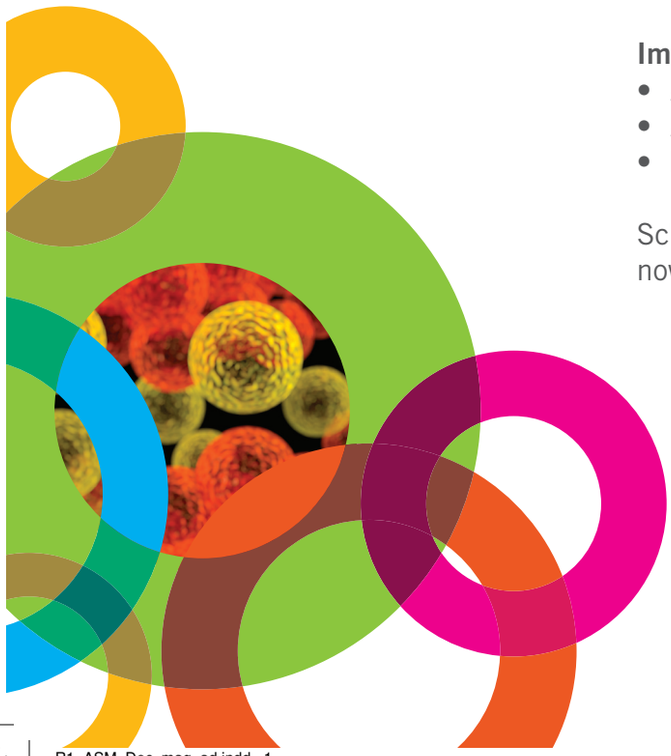

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