**Echinococcosis: A chronic, complex, and still neglected disease**

**Introduction:** Echinococcosis is a serious chronic neglected disease with poor prognosis and high mortality if managed inadequately. More than two million cases of cystic echinococcosis (CE) occur globally each year. Both CE and alveolar echinococcosis (AE) has been reported from the Arabian Gulf however, CE is more prevalent in the Middle East (ME) compared to AE which is restricted to the northern hemisphere. Most information on the prevalence and incidence of CE in the ME is based on case reports using serology and ultrasonography for conducting population studies.

**Methodology:** A systematic search was conducted in electronic databases to get information on the Arabian Gulf and the hospital registry was screened to get information on echinococcosis in Kuwait. All the studies were examined in order to select available epidemiological and case report studies.

**Results:** A total of 31 studies met the eligibility criteria and the data was extracted. *E. granulosus* infection was the main infection in the Arabian Gulf. Epidemiological factors associated with increased risk of *E. granulosus* infection in definite hosts including the dogs and camel, lack of public health awareness and access to health facility were the main risk factors determined. The key factors associated with CE infection in intermediate hosts were related to the hosts’ age and the level of environmental contamination with parasite eggs. In addition, a series of atypical CE cases with a number of unusual radiological appearances and sites, were reported from Kuwait. We briefly discuss the pathology of hydatid disease and its complications. A typical and an atypical case of CE from Kuwait is presented in relation to WHO-IWGE classification of CE for choosing from four appropriate treatment and management options. In addition, some of the recent concerns and future challenges in the diagnosis, treatment, and management of echinococcosis will be discussed.
Development and application of a simple LC-MS method for the determination of plasma dolutegravir concentrations

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Background:
Dolutegravir is a second-generation HIV-1 integrase inhibitor that is highly potent against both wild-type and drug-resistant HIV-1 strains. The quantification of dolutegravir in human plasma is important to support clinical studies. Dolutegravir was just approved in April 2014 in Japan. Therefore, pharmacokinetic study of dolutegravir for Japanese is still not clear. We intended to develop a conventional method for determining plasma dolutegravir concentrations and compare plasma dolutegravir concentrations of Japanese HIV-1 infected patients with that of foreign patients.

Methods:
We used a Waters Alliance 2695 HPLC and a Micromass ZQ-2000 MS, controlled with MassLynx version 4.0 software. Our method involves rapid liquid-liquid drug extraction from plasma and use of gradient elution on a reversed-phase C18 column. We recruited 31 Japanese HIV-1-infected patients who were treated with dolutegravir containing regimen in Japan. All patients had been administered with 50 mg dolutegravir once daily in combination with other antiretrovirals.

Results:
The established LC-MS method was validated by estimating the precision and accuracy for inter- and intra-day analysis in the concentration range of 79-4012 ng/ml. The calibration curve was linear in this range. Relative standard deviations of both inter- and intraday assays were less than 4.3%. In this study, mean dolutegravir plasma concentration for Japanese patients at trough was 0.71±0.50 ug/ml. Mean dolutegravir concentration at peak was 2.89±0.74 ug/ml. These values were similar with dolutegravir concentrations seen in foreign HIV-1-infected patients’ trials.

Conclusion:
Our LC-MS method can be used conveniently in clinical routine application and enables the study on the pharmacokinetics of dolutegravir in conventional hospital laboratories. In general, body build of Japanese is poor in comparison with Caucasian. So high plasma dolutegravir concentrations may result in dose reduction for Japanese. However, our data showed that the dose adjustment of dolutegravir is not also required for Japanese HIV-1-infected patients.
Antibiotic resistance of Enteropathogenic Escherichia coli isolated from diarrheal children in Milad hospital during 2012-13

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Aim and background: In this study drug susceptibility of enteropathogenic Escherichia coli (EPEC) was detected in diarrheal patients. The increase in antibiotic resistance in pathogenic bacteria, especially in children, is considered as one of the world's health problems. The aim of this study was to evaluate the antibiotic resistance in EPEC which isolated from children with diarrhea admitted to the Milad hospital in Tehran.

Methods: This study was conducted from March 2011 through March 2013. Fecal or rectal swab specimens from children (<5 years) were collected and then processed, using standard methods, to detect Salmonella spp., shigella spp., and EPEC. The antibiograms of the entropathogens were determined using the disk diffusion method. The E coli positive cultures were set for serological test, by slide agglutination test, according to BioRad procedures, using EPEC polyvalent O antisera for EPEC, (I, II, III, and IV) separately.

Results: A total of 7321 samples were processed: 433 enteric pathogen were isolated from samples. From 433 positive samples, 146 came out positive for EPEC. Based on the results of antimicrobial testing resistance to: ampicillin, cefixime, Co-trimoxazole, Nalidixic acid, ciprofloxacin, ceftriaxone, and chloramphenicol were 82.19%, 79.45%, 64.38%, 59.5%, 36.98%, 34.93%, 13.6% respectively. The highest number of EPEC isolated belonged to polyvalent serogroup was 3 serogroup (85%), 4:3%, 2:5%, and 1:7%.

Conclusion: Highest sensitivity was to chloramphenicol in this study, but in clinical practice, it has serious side effects. Therefore it suggest that application of antibiogram test is necessary before antibiotic prescription for successful treatment and prevention of diarrhea caused by multi-drug resistance agents.

Key words: Diarrhea, EPEC, Antibiogram, susceptibility, disk diffusion
Profile Characteristic Patterns of Fever Associated with Microorganism Culture
Results at Internal Medicine Department M. Hosein Hospital Palembang, Indonesia

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Background
Fever are the main symptoms of the disease caused by bacterial infection. In addition, infectious diseases are still a problem in Indonesia and other developing countries, particularly infectious diseases caused by bacteria. There are many criteria for diagnosing the underlying febrile disease caused by bacterial infection, one of which became the gold standard is culture microorganism. Therefore it is necessary to do research on the relationship characteristic pattern of fever due to bacterial infection and bacterial causes.

Purpose
This study aimed to identify characteristic pattern of fever due to bacterial infection and bacterial causes in Department of Internal Medicine, Dr. Moh. Hoesin Hospital Palembang.

Methods
This study took a sample of the three populations disease, which typhoid fever, pneumonia and urinary tract infections that have symptoms of fever and its culture has been examined in Palembang RSMH inpatient period July 2009 - June 2010. This study is descriptive in order to determine the characteristics of patients with fever and its culture and its relationship results. Data collection is done by taking the data from medical records.

Results
Based on the results of this study found that 45 typhoid in 109 samples, 31 of pneumonia and urinary tract infections in 33 samples. For ages the highest frequency in the age group is 18-30 years, the number of men more than women on urinary tract infections, balanced in typhoid fever and the number of women more than men in pneumonia. Frequencies distribution of body temperature and leukocyte count showed similarities to the three diseases, namely in the range of 37.5 to 38.0 and 10000-15000/mm³. Most cultures are Salmonelathypii on typhoid which 31 cases (68.9%), pneumonia cases that Klebsiellapneumoniae in 10 cases (32.1%) and Escheria coli in urinary tract infections 12 cases (36.3%).

Conclusion
The conclusion of this study is that the patients who treated in hospital cause by fever either men or women have to check microorganism culture for getting accurate therapy.

Keyword
fever, microorganism

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IN VIVO EFFECT OF SOME MALAYSIAN MEDICINAL PLANT EXTRACTS ON THE TOXOPLASMA GONDII TACHYZOITES

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Abstract
Toxoplasmosis drugs have the longest history and are still the first choice for most conditions. Alternative drugs such as Co-trimoxazole and Tetracycline have been tried and acclaimed successful. The lack of general acceptance, however, is an indication that the results are not very convincing. A wide range of antibiotics is urgently needed for patients with drug reaction or resistance problems. The anti-toxoplasmic activity of water and ethanol extracts as well as some Malaysian medicinal plants (Kaempferia galanga, Azadirachta indica, Piper Sarmentosum and Curcuma longa), were evaluated in murine models of intraperitoneal infection using the RH strain of Toxoplasma gondii. Female mice were infected with 2×10^2 tachyzoites/ml, and then treated intraperitoneally with the Malaysian medicinal plants at 100 and 200 mg/kg/day for seven days. The tested extracts reduced the mean number of tachyzoites present in the peritoneal fluid of the experimental mice. The most effective extract was Curcuma longa ethanol extract which showed a 98.6% and 99.2% inhibition of the growth of Toxoplasma tachyzoites in 100 and 200 doses respectively compared to the control infected untreated mice.

Keywords: Anti-toxoplasmic activity, Kaempferia galanga, Azadirachta indica, Piper Sarmentosum and Curcuma longa, Toxoplasma gondii, RH strain
Self-adjuvanting promiscuous peptide of *Mycobacterium tuberculosis* augments polyfunctional Th17 cells and evokes better memory T cell response than BCG

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Abstract

Background: Vaccines have been successful in worldwide eradication of dreaded diseases like smallpox, diphtheria, tetanus, yellow fever, whooping cough, polio, and measles. Unfortunately, such triumph has not been achieved in controlling tuberculosis (TB) globally. Bacillus Calmette Guérin (BCG) is the only available vaccine against TB. Paradoxically, BCG has deciphered successful results in the Western population but has failed in TB-endemic areas. Hence, it is quite crucial to understand the immunity responsible for controlling *Mycobacterium tuberculosis* infection and factors responsible for the failure of BCG in TB-endemic countries. Consequently, introducing radical changes in the vaccines that would impart protection in the populations where BCG has failed. One of the main reasons considered for BCG failure in TB-endemic areas is impediment by environmental mycobacteria in its processing by antigen presenting cells and generation of memory T-cell response.

Methods: The peripheral blood mononuclear cells of sputum positive pulmonary TB patients and their house-hold contacts were separated by ficoll-hypaque gradient method. The cells were cultured with L91 and proliferation was monitored by CFSE-dye dilution assay and phenotypic markers by flow cytometry using fluorochrome tagged appropriate antibodies and their isotype-matched controls.

Results: Keeping in view the shortcomings of BCG, we developed a unique lipopeptide (L91) by linking the promiscuous peptide (sequence 91-110) of 16 kDa antigen of *Mycobacterium tuberculosis* to Toll-Like Receptor-2 agonist Pam2Cys. L91 does not require extensive antigen processing and targets and activate dendritic cells. This is evidenced by the fact that L91 significantly improved the activation and proliferation of polyfunctional Th1 and Th17 cells of the TB patients and their house-hold contacts. Furthermore, L91 surmounts the barrier of major histocompatibility complex polymorphism. Importantly, this peptide has self-adjuvanting property and induces enduring memory T cell response, which is significantly better than BCG.

Conclusion: L91 can be a potent future vaccine candidate against tuberculosis in TB-endemic and non-endemic zones.

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Increasing Prevalence of Candiduria in a Tertiary Care Hospital of Kabul

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ABSTRACT

Background: The prevalence of Candida has been on rise worldwide. Urinary tract infections (UTIs) with Candida species are becoming frequent particularly in hospitalized patients. Physicians face dilemma in differentiating colonization from true candiduria. Often the line between Candida colonization and infection is blurred. Diagnosis typically depends on the discovery of pyuria with high colony Candida counts in the urine.

Objective: The aim of the study was to find out the frequency of Candida species among uropathogens, their speciation and to determine the cases of candiduria having pyuria.

Material and Methods: Urine samples of both genders and of all ages, suspected of having UTI, submitted for culture between 2008 and 2013, were analyzed in a tertiary care hospital. The samples were collected in a sterile leak proof container with screw capped lids and transported immediately to microbiology laboratory. Urine wet mount examination was done to look for the presence of pus cells, red blood cells, casts, or any bacterial or fungal elements. The urine samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) and CHROM agar by calibrated wire loop technique delivering 0.001ml of urine as per standard protocol for urine culture. The culture plates were incubated aerobically at 37°C for 24 to 48 hours. Candida species isolated on culture plates with colony count \( \geq 10,000 \text{ CFU/ml} \) were considered significant and were subjected to germ tube test for the direct identification of \textit{Candida albicans}.

Results: A total of 647 (3.02\%) Candida species were isolated from 21,455 urine samples. Among them \textit{Candida albicans} 423 (65.4\%), were predominant compared to non-\textit{albicans} Candida species 224 (36.6\%). There has been an increase in the frequency of candiduria over the period of six years. The rate of isolates of Candida species were more in females, 397 (61.4\%) than in males 250 (38.6\%). The isolation rates of Candida were 358/647 (55.3\%) in age group up to 18 years. Out of all cases of candiduria, 350 (54.1\%) had pyuria.

Conclusion: The present study reiterates the prevalence of Candida species among UTIs which has been increasing every year. Physicians need to confirm the infection by a second sterile urine sample and correlate the findings with symptoms of UTI and presence of pyuria. Asymptomatic candiduria is usually benign, and does not require antifungal therapy. Many studies have demonstrated that non-\textit{albicans} Candida are more resistant to anti-fungal drugs. Therefore, the species identification of Candida isolates along with their antifungal susceptibility pattern can help the clinicians in better treating the patients with candiduria.

Keywords: Candiduria, Non-\textit{albicans} Candida, Urinary tract infection
Speedy and accurate diagnosis of tuberculosis and rifampicin resistance with a new tool

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Abstract

Background: Detecting tuberculosis (TB) rapidly and identifying drug resistance on the spot are crucial to improve care of patients and avoid transmission in the community. Conventional tests can take weeks to months to complete. A new diagnostic assay, Xpert MTB/RIF, can deliver a TB test result within two hours. The Xpert test can also detect resistance to rifampicin, one of the first-line drugs most commonly used to treat the disease. This is particularly important in areas where drug-resistant TB is common.

Objective: The aim of this study was to evaluate the diagnostic performance of the Xpert MTB/RIF assay and compare the results with those of smear microscopy in patients with suspected pulmonary tuberculosis.

Methods: Between Jan 1, 2013 and Jun 30, 2014, the Xpert MTB/RIF assay using sputum was requested in 100 patients with suspicion of pulmonary TB. The median age of these patients was 31 years and 67 were female. Untreated sputum samples were vortexed for 5 minutes and split into 2 aliquots; one was processed for standard sputum microscopy for Acid Fast Bacilli (AFB) after Ziehl-Neelsen staining and second was sent to PCR laboratory for the Xpert MTB/RIF assay, an automated real-time nucleic acid amplification technology for rapid and concurrent detection of TB and Rifampicin resistance. The Xpert assay was performed and interpreted according to the manufacturer’s instructions.

Results: A total of 100 sputum samples were analyzed. Results of smear microscopy were compared against diagnostic performance of Xpert MTB/RIF assay. Out of all samples submitted for the Xpert assay, 64 were positive for Mycobacterium tuberculosis (MTB), while examining the samples under the microscope turned up only 22 cases. Thus, out of 64 there were 42 (65.6%) cases which were negative on microscopy but positive for MTB. In other words, around 2/3rd of cases could have been undiagnosed if only microscopy would have been relied upon. Hence, Xpert detected about 3-fold more tuberculosis cases than smear microscopy but with equal rapidity. Among patients who were negative for AFB, the results of the Xpert assay were negative for TB with 100% accuracy. Amongst those positive for MTB, 28 (43.75%) were resistant to the drug rifampicin.

Conclusions: Xpert MTB/RIF can detect three times the number of TB cases identified by examining specimens under the microscope, a preliminary method for diagnosis which must be verified by the culture test. In addition, Xpert test could provide a quicker way to identify patients who need alternative treatment regimens. This is very important and could potentially save lives as well as help to limit the rise of drug resistant TB.

Key Words: Rapid diagnosis, Real-time PCR, TB, Xpert MTB/RIF
Evaluation of Immune Response to Hepatitis B Vaccine in Egyptian Children

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Background
Hepatitis B is considered a major health problem in Egypt and worldwide that can lead to chronic hepatitis, hepatic cirrhosis, hepatocellular carcinoma and liver failure. Approximately 350 million people worldwide had chronic HBV infection and that 1 million persons die each year from it (Kao and Chen, 2000).

A study in Egypt found that the prevalence rate of Hepatitis B was moderately high (10.1%); higher in Upper Egypt than Lower Egypt, in young adults males than females (Sherif et al., 2005). In 1992, WHO recommended that all countries integrate hepatitis B vaccine (recombinant DNA-based vaccine, targeting the HBV major surface protein) into national immunization programs.

MATERIALS AND METHODS
The present study was conducted on 182 children (110 males and 72 females categorized into eight age groups from 2-10 y/o). Children had received HBV vaccine at 2, 4, and 6 months of age, according to the vaccine schedule of the Egypt, they were subjected to detailed clinical history and full clinical examination to exclude HBV infection, those suffering from chronic illness, on longterm steroid treatment or children of mothers infected with hepatitis B during pregnancy were excluded.

We evaluated the long-term efficacy of infant hepatitis B vaccination program in preventing hepatitis B virus infection, and to assess its impact on the incidence of hepatitis B in children. Serum samples from each participant were tested for the quantitative determination of anti-HBs antibody using enzyme linked immunosorbent assay (ELISA) as well as qualitative ELISA testing for HBsAg and HbcAb.

The study showed that the efficacy of hepatitis B vaccine, as evidenced by presence of protective levels of HbsAb, is 88.5%.

HBsAg was not detected in any participant. The protective rate for the 2-6 years age groups was 100% while the protective rate for the 7–10 years age group was less.

Results
We concluded that hepatitis B vaccine efficacy is high in an immunocomptent population, and that a booster vaccine dose is not necessary before 6 years of age.

Gender difference was not found.

CONCLUSION
Hepatitis B vaccination program in Egyptian children appears to be initially effective in the first few years (2-6 years) following vaccination, as evidenced by high levels of anti-HBs antibody, together with absence of serological evidence of infection or carrier states.

The decrease in antibody levels in older children (7-10 years), although expected, is not alarming, since exposure to infection would most likely trigger an anamnestic response that would lead to a spike in protective antibody levels. Moreover, the majority of older children retained a level of antibody which, although lower, was still in the protective range.
Prevalence and characterization of carbapenem- and polymyxin-resistant \textit{Acinetobacter baumannii} strains from Terengganu, Malaysia

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Background: \textit{Acinetobacter baumannii} has been implicated in various nosocomial infections and the therapeutic option for these \textit{Acinetobacter} infections is increasingly limited due to its uncanny ability to acquire antimicrobial resistance. Carbapenems was the drug of choice for the treatment of \textit{A. baumannii} infections but the efficacy of carbapenems has been increasingly compromised due to the spread of carbapenem-resistant clones. This led to the use of polymyxins as the drug of “last resort” but unfortunately, \textit{A. baumannii} strains that are resistant to polymyxins have been reported. The aim of this study is to determine the resistance profiles and genetic diversity of \textit{A. baumannii} isolated from the main tertiary hospital in the state of Terengganu, Malaysia.

Methods: Fifty four clinical strains of \textit{A. baumannii} were collected throughout 2011 from sporadic cases of infection. Antibiotic susceptibility profiles of the strains were investigated by Kirby-Bauer disc diffusion method. Antibiotics used were gentamicin, tobramycin, amikacin, ciprofloxacin, levoflaxacin, piperacillin-tazobactam, cefotaxime, ceftazidime, cefepime, ampicillin-sulbactam, tetracycline, and doxycycline. Minimal inhibitory concentration (MIC) to carbapenems and polymyxin B were determined using M.I.C. Evaluator (M.I.C.E) strips (Oxoid, UK) and agar dilution method, respectively. Guidelines from the Clinical and Laboratory Standard Institute (CLSI, 2013) were used to interpret the diameters of the inhibition zones produced and analyze the MIC data obtained. Pulsed-field gel electrophoresis (PFGE) was carried out on all 54 strains using previously published protocols on a CHEF-DRIII system (BioRad).

Results: High percentages (>50%) of antibiotic resistance were observed for all antibiotics tested. Most of the strains (87%) were resistant to tetracycline while only 57.4% were resistant to amikacin. The strains also showed high resistance to carbapenems: 77.8% to meropenem and 74.1% to imipenem. The majority of the carbapenem-resistant strains showed MIC values of $>$ 32 $\mu$g/mL. Of the 54 strains, 39 (or 72.2%) could be categorized as multidrug resistant (MDR) (i.e., resistant to more than three classes of antibiotics). All MDR strains were resistant to meropenem and imipenem. Fourteen (or 25.9%) strains were categorized as polymyxin resistant. Four of these strains had MIC values of $>$ 128 $\mu$g/mL for polymyxin B. All 14 polymyxin-resistant strains could also be categorized as extensive drug resistant (XDR). PFGE profiles of the 54 strains showed that the carbapenem-resistant strains were clustered into four groups (at identity levels $>$80%) whereas no clustering was observed for the polymyxin-resistant strains.

Conclusions: High incidence of resistance to carbapenems (~ 75%) and the detection of XDR polymyxin-resistant strains (25.9% prevalence) of \textit{A. baumannii} from the main tertiary hospital in Terengganu, Malaysia, is a cause of concern. The overuse of carbapenems and polymyxins in the hospital may have led to the occurrence and spread of XDR \textit{A. baumannii}.

(499 words)
Absence of cytomegalovirus in transcriptome and high-coverage DNA sequencing argues against antiviral treatment of human glioblastoma multiforme.

Viruses cause 10–15% of all human cancers. Massively parallel sequencing has recently proved effective for uncovering novel viruses and virus–tumour associations. We screened a diverse landscape of human cancer, encompassing 4,433 tumours and 19 cancer types, for known and novel expressed viruses based on >700 billion transcriptome sequencing reads from The Cancer Genome Atlas Research Network. The resulting map confirms and extends current knowledge. Our analysis argues strongly against viral aetiology in several cancers where this has frequently been proposed. For example, we were unable to detect any cytomegalovirus (CMV) in 22.8 billion sequencing reads of mRNA from 167 glioblastoma multiforme tumors. We also utilized deep-coverage whole-genome sequencing data to detect latent CMV DNA and to assess the relative proportions of viral and human DNA. We did not find traces of CMV in 52.6 billion DNA sequencing reads from 34 glioblastomas. By statistical analysis, we conclude that should the virus be present in these tumors, the average CMV level does not exceed one virus per 240,000 tumor cells (99% CI). These findings together strongly argue against current antiviral treatment of glioblastoma multiforme.
Background: Drug resistance in bacteria is increasing, while the pace at which new antibiotics are being produced is slowing. There has been an increasing interest in herbal extracts and their essential oils during recent years because of the need of new therapies against microbes to develop natural, safe, inexpensive and effective alternative. We aimed to determine the antibacterial activity of some medicinal herb extracts.

Methods: Five herbs (namely: mint, green tea, ginger, garlic and aloe vera) were tested against methicillin resistance *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (*P. aeruginosa*) for their antibacterial activity using: Broth dilution, Agar-well diffusion and Synergy assays.

Results: The examined bacteria were sensitive to the five tested herbal extracts with varying degrees. The broth dilution assay yielded that garlic extract had the maximum activity while aloe vera had the minimum activity against all the tested bacteria with significant difference. The minimum inhibitory concentration (MICs) of mint, green tea, ginger, garlic and aloe vera against *P. aeruginosa* were 75, 75, 150, 37.5 and 300, respectively, while corresponding figures against MRSA were 150, 150, 150, 18.75 and 300, respectively. Garlic extract had a maximum inhibition zone diameter against *P. aeruginosa* and MRSA (15 mm and 45 mm, respectively). The result also showed that, although the AV alone had a minimum antibacterial activity against examined bacteria but had a synergic effect with Garlic and with Ginger against *P. aeruginosa* and with Garlic and green tea against MRSA. The majority of combinations displayed an indifference effects. Aloe vera showed additive effects to garlic, ginger and green tea.

Conclusion: Herbal extracts can provide an adequate degree of protection against the tested organisms, where garlic was most effective and aloe vera was least effective. Garlic can be utilized for the development of broad spectrum antibacterial agents as it has wide spectrum antibacterial activity. The use of herbal extracts in combination is recommended to reduce the concentration used and to widen the spectrum activity.
**Bacterial infections in the Hematology Unit, Alexandria Main University Hospital, Egypt**

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**Background:** Healthcare–associated infections (HAIs) are due to different types of organisms and these are increasing in their resistance to antibiotics. The treatment of these infections is one of the most difficult processes to help cure of those patients. The present study aimed to estimate the incidence of HAIs, identify bacterial profile and perform antibiogram of isolated strains among hematology patients.

**Methods:** The study was carried out on 66 adult patients admitted to the Hematology Unit, Alexandria Main University Hospital, during a 12 months period. Samples included blood, sputum, urine, throat swab, and skin swab. All samples were subjected to full bacteriological investigations and antimicrobial susceptibility testing by disc diffusion method according to the CLSI guidelines.

**Results:** The 66 patients developed 92 infectious episodes. The sites of HAIs were mostly blood stream infections (BSIs) 82.6%, followed by pneumonia (13%). There was a predominance of the Gram-positive cocci which represented 52% of isolates, Gram-negative bacilli represented 44% while Candida spp represented 4%. The most common isolate overall was *coagulase negative staphylococci* (30%) followed by *Staphylococcus aureus* (22%) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (14%), while *Klebsiella pneumonia* (*K. pneumonia*), *Escherichia coli* (*E. coli*) and *Acinetobacter baumannii* represented 8% each, followed by *Citrobacter spp* 6%. Regarding the antibiotic resistance, 90% of *S. aureus* were methicillin resistant *Staphylococcus aureus* (MRSA), while vancomycin resistance was observed among 9.1% of isolates. All *K. pneumoniae* and *E. coli* isolates were identified as Extended-Spectrum β-Lactamase (ESBL) producing organisms. *P. aeruginosa* were resistant to most antibacterials tested; 100% were resistant to ceftazidime and cefoperazone, followed by cefepime (85.7%), amikacin (71.4%), gentamicin (42.8%) and piperacillin (28.6%), while showed less resistance to imipenem and levofloxacin (14.3% each).

**Conclusion:** Blood stream infections are the most common HAIs in hematology patients. Isolated organisms are multi-drug resistant, predominantly Gram-positive pathogens with high incidence of MRSA, ESBLs and multidrug resistant *P. aeruginosa*.
Molecular detection of \( bla_{IMP-1, 2} \), \( bla_{KMH-1} \), \( bla_{SPM-1} \) genes in metalo-\( \beta \)-lactamase producer \( Pseudomonas aeruginosa \) isolates in Ghotbeddin Shirazi Burn Hospital (Shiraz 2012-3)

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**Background:** *Pseudomonas aeruginosa* is a Gram-negative bacterium that is considered as an important nosocomial infection. It is resistant to many antibiotics mainly because of the low degree of its outer membrane permeability, reduced levels of drug accumulation due to active efflux pumps and producing \( \beta \)-lactamases enzymes. In Ambler classification, \( \beta \)-lactamase divided into A-D classes. Group B unlike the others has metal dependence, so they are named metalo beta-lactamase (MBLs). Due to the importance of MBLs in drug resistance particularly in burn patients, we aimed to determine the prevalence of these enzymes in *P. aeruginosa* isolates.

**Methods:** Totally, 210 samples were collected from burn patients hospitalized at Ghotbeddin Shirazi Burn Hospital, during September 2012 to November 2013. Samples were obtained from wound of patients with \( \geq \) 48 hour hospitalization. Isolates were tested for *P. aeruginosa* by standard bacteriological tests. Confirmation of the primary detection of *P. aeruginosa* isolates was performed by PCR for 16S rRNA region. Susceptibility test for confirmed isolates was performed using disc diffusion method (Kirby-Bauer), recommended by CLSI guideline for antibiotics Pipracilin-Tazobactam, Imipenem, Meropenem, Amikacin, Ceftazidim, Cefotaxim, Gentamicin, Ciprofloxacin, Cefepim, Chloramfenicol and Tobramycin. The production of MBL was detected by the zone size enhancement with EDTA impregnated imipenem disc compared with imipenem alone. Finally, PCR was performed using specific primers for \( bla_{IMP-1, 2} \), \( bla_{SPM-1} \), and \( bla_{KMH-1} \) genes.

**Results:** Fifty-five (26.19%) out of 210 samples, were detected as *P. aeruginosa* using conventional methods in which 47 (85.45%) were confirmed with molecular method. All of the confirmed isolates were resistant to all antimicrobial agents except for colistin. Twenty-six (61.9%) isolates were detected as MBLs producer. The frequency of \( IMP-1 \), \( IMP-2 \), \( KHM-1 \) and \( SPM-1 \) genes in MBLs isolates were 0 (0%), 5 (19.23%), 4 (15.38%) and 0 (0%), respectively.

**Conclusion:** Because of the high prevalence of MBL *pseudomonas* spp. in burn patients, it is highly recommended to change the choice of drugs with MBLs resistant drugs or use synergy inhibitor MBL compounds with these drugs. Each medical center should have an antibacterial pattern that is updated regularly to conquer the prevalence of resistant in their zone of infection.

**Keywords:** Metalo \( \beta \)-lactam, *Pseudomonas aeruginosa*, Burn patients, Shiraz, Iran
Title:
Association of Colistin with Nephrotoxicity

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Background: The increasing trend of extensively drug-resistant (XDR) gram negative infections among ICU patients has resulted in the reappraisal of Colistin as a last resort drug. Previous reports highlighted nephrotoxicity as the main adverse effect related to Colistin usage. This study aims to determine the association of Colistin with nephrotoxicity.

Methods: The medical records of ICU patients on Colistin in Hospital Serdang and Hospital Sungai Buloh from 2010 to 2012 were retrospectively reviewed. Patient demographic data, treatment characteristic and microbiological profile data were documented and studied for their involvement on nephrotoxicity. Nephrotoxicity was determined based on RIFLE criteria.

Results: A total of 100 patients between 6 and 83 years of age were included in the study. Colistin indication was mainly in pneumonia (47%) with Acinetobacter baumannii as the leading causative organism (71%). Nephrotoxicity was developed in 23% of the study population. Mean age for nephrotoxicity group was 48.17±21.32 (range: 9-75) years and without nephrotoxicity group was 44.69±19.08 (range: 6-83) years. Median daily dose and cumulative dose for nephrotoxicity group were 3.0 MU (IQR: 2.0; range 1-6) and 17.6 MU (IQR: 22.0; range 2-102) respectively, while for without nephrotoxicity group were 3.0 MU (IQR: 4.0; range 1-12) and 18.0 MU (IQR: 34.0; range 2-180). Median duration for nephrotoxicity group was 5.0 days (IQR: 7.0; range: 1-17) and for without nephrotoxicity group was 7.0 days (IQR: 4.0; range: 1-30). Patient age, dosage and duration of Colistin had no significant association with nephrotoxicity by Simple Logistic Regression model.

Conclusion: Colistin is relatively safe with appropriate dose and duration with close monitoring of renal function. Although comorbidities and concomitant use of other nephrotoxic drugs were not included in this study, they may contribute to nephrotoxicity and should be monitored accordingly.
The prevalence and susceptibility pattern of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) in abscesses in north western province Sri Lanka

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BACKGROUND:
Community-associated methicillin resistant Staphylococcus aureus (CA-MRSA) infections are increasing globally. The most common of these infections present as skin abscesses. The objectives of this study were to determine the prevalence of CA-MRSA in abscesses and to determine antibiotic sensitivity patterns of the CA-MRSA isolates.

METHODS:
We conducted this retrospective study from July 2013 to July 2014 in the Microbiology Department District General Hospital Chilaw Sri Lanka. Aspirated pus from abscesses was received from surgical units for culture to determine the causative agent, and antibiotic sensitivity pattern. Standard isolation and identification was carried out. Antibiotic susceptibility test was done by disk diffusion method. Cefoxitin 30ug and Oxacillin 1ug was used for methicillin resistance detection. Lab records were reviewed. Inducible Clindamycin resistance was tested by double disk diffusion method. Chi-Square as well as Fisher's exact test was used to compare categorical variables using SPSS 13 for Windows®.

RESULTS:
During the study period 632 pus cultures were processed. One hundred and fifty six (156) isolates were identified as Staphylococcus aureus. Fifty six (56) isolates were Methicillin Sensitive Staphylococcus aureus (MSSA). All 100 MRSA isolates were identified as CA-MRSA by their resistance patterns. Hence Community associated MRSA prevalence in this study was 64%. Of these sixty percent (60%) of them were females and 65% of them were adults. All were sensitive to vancomycin. Susceptibility of CA- MRSA isolates to fusidicacid and cotrimoxazole and gentamicin was 93% and 86% and 80% respectively. Susceptibility to ciprofloxacin was low in CA-MRSA 65%. Majority were resistant to erythromycin 69 % (69/100) and clindamycin 53%. Inducible clindamicin resistance was 42%.

CONCLUSIONS:
Very high prevalence of CA-MRSA from skin abscesses was observed in this study. CA-MRSA isolates showed good susceptibility to fusidicacid and cotrimoxazole. But clindamycin resistance was high. Therefore our recommendation is to use cotrimoxazole as a treatment for abscesses when antibiotics are indicated.
Phenotypic and Genotypic Characterization of Carbapenemases producing Enterobacteriaceae.

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Background: Carbapenems remain as the reliable and effective antibiotic options in the treatment of multi-drug resistant gram negative organisms such as Extended Spectrum Beta-lactamases (ESBL) and AmpC enzymes producing Enterobacteriaceae. However, recently, there are increasing reports of Carbapenemase producing Enterobacteriaceae (CRE), compromising the effectiveness and usage of these antibiotics in clinical settings. We are reporting a two-year nationwide study on the phenotypic detection of CRE among the bacteria isolates in hospitals in Malaysia and genotyping study of carbapenemases enzymes particularly, Class B, metallo-beta-lactamase (MBL) enzymes (New Delhi Metallo-beta lactamase gene), Class A (KPC gene) and class D(carbapenem oxacillinases or (Oxa-48 gene).

Method: A total of 1725 isolates were screened for carbapenemases production. Identification of organisms was performed using API 20E or Vitek2 Compact system (bioMe´rieux, France). Enterobacteriaceae isolates exhibiting intermediate or resistant phenotype to carbapenem drugs by disc diffusion were further verified using E-test to imipenem, meropenem and ertapenem and screening for carbapenemases production by Modified Hodge test (MHT). Synergy test using ethylene diamine-tetra-acetic-acid (EDTA) disks as well as combined test using EDTA with ertapenem drug were also performed. The presence of carbapenemases resistance genes was established by PCR using specific primer pairs.

Result: Majority of the isolates were *Klebsiella pneumoniae*. Out of 1725 organism tested, 1268 (73.5%) were phenotypically detected as resistant to carbapenem drug. Thirteen percent (n=225) were sensitive to all carbapenem while, 12.4% (n=213) were only resistant to one antibiotic mainly ertapenem due to production of β-lactamases enzymes combined with porin loss. Nineteen cultured isolates were mixed growth and were not further analyzed.

Based on PCR analysis for CRE cases, 78.6% (n=997) harboured blaNDM-1 gene, 0.6% (n=6) possessed blaKPC gene, 3.9% (n=50) has blaIMP gene, 0.4% (n=5) carried blaVIM and 2.8% (n=35) was positive for blaOXA-48 gene. A total of 13.7% (n=175) do not harbor any of the genes tested. Based on this study, MHT proved as a reliable method for detection of CRE with sensitivity and specificity of 100% and 96.7%, respectively. Combined test and synergy test also demonstrated a sensitivity of 100% however the specificity was 96.7% and 93.3% respectively.

Conclusion: Enterobacteriaceae harbouring blaNDM-1 is endemic in our health care settings. Other types of carbapenemases are also reported. Hence, it is important for laboratories to be vigilant about the detection of these organisms. Due to the high prevalence of Class B carbapenemases enzymes among the isolates, Modified Hodge test, EDTA synergy or in combination with carbapenem, are simple yet sensitive methods that can be used for phenotypic detection of CRE in laboratory settings.
Isolation and identification of medically important yeasts and yeast-like fungi (Cryptococcus, Candida and Rhodotorula spp.) from bird droppings in Iran.

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Background: Yeast and yeast-like fungi such as species of Cryptococcus, Candida and Rhodotorula are emerging as important human pathogens in immunocompromised and immunocompetent patients. The purpose of this study was isolation and identification of three medically important yeast genera from avian manure in Iran. Methods: In order to fungal isolation, the excreta of pigeon, parrot, love bird, canary and poultry were cultured on the culture media, Sabouraud dextrose agar and Brain heart infusion agar, and identification was carried out via direct examination of the samples and by using urease and carbohydrate assimilation test (API 20C AUX). Results: Out of 250 manure samples, 70 (28%) different yeast and mold fungi were recovered. Cryptococcus uniguttulatus was the most frequently isolated species representing 5.72% of isolates, followed by four other Cryptococcus species including C. laurentii (4.28%), C. neoformans (4%), C. albidus (2.86%) and C. hmicola (1.43) as well as 10 other fungal genera. Conclusion: The results showed that medically important yeasts are scattering in environment of Iran and immunocompromised persons, particularly HIV positive and AIDS patients potentially are in danger of these contaminated excreta.
Distribution of Metallo-beta-lactamase (MβL) genes among Acinetobacter baumannii isolated from clinical samples from Shiraz, South-west of Iran

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Background: Metallo-beta-lactamase (MβL) enzymes production is the most important resistance mechanism against carbapenems in some bacteria including A. baumannii. The aims of this study were to determine the antimicrobial susceptibility and the prevalence of Metallo-beta-lactamase (MβL) among carbapenem-resistant isolates of Acinetobacter baumannii.

Methods: In a cross-sectional study from October 2012 to April 2013, 98 isolates were identified as A. baumannii using Microgen™ kits & blaOXA-51-like PCR assay. These isolates were tested for antimicrobial susceptibilities by disk diffusion method according to CLSI guidelines. Carbapenem-resistant isolates were further detected phenotypically by E-Test MβL and subsequently positive MβL isolates were detected by PCR.

Results: From a total of 98 isolates of A. baumannii 96 (98%) isolates were detected as Carbapenem-resistant by E-Test. Highest sensitivity to the tested antibiotic with 46.9% and 42.9% were observed to polymixin B and colistin, respectively. Of 96 carbapenem-resistant isolates, 43 were phenotypically positive for MβL; out of 43 isolates, 37 confirmed the presence of MβL genes by PCR.

Conclusion: High antibiotic resistance and MβL production in Acinetobacter isolates were observed. MDR among A. baumannii isolates are increasingly observed, since all MβL producing isolates were MDR. This is a serious consequence of the wide use of antibiotics as empirical therapy in nosocomial infections.

Keywords: Carbapenem, Acinetobacter baumannii, Metallo beta-lactamase, Antibiotic resistance, E-Test
**Resistance mechanisms in carbapenem-resistant Enterobactericeae (CRE) isolated in Singapore hospitals**

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**Background**

Molecular investigative tools have facilitated the identification of sophisticated antimicrobial resistance mechanisms in bacteria, such as carbapenemase production. True extent of carbapenemase-producing CRE (CP-CRE) burden in Singapore is unknown though there has been an increasing trend in patients with CP-CRE over the past four years. The carbapenemase producing enterobacteriaceae in Singapore (CaPES) study is aimed at documenting local CP-CRE epidemiology.

**Methods**

CaPES is a prospective cohort study involving 6 public hospitals in Singapore. Since December 2013, consenting CRE positive patients are being recruited and followed up for 30 days if infected and 1 year if colonized with CP-CRE. We present the descriptive epidemiology of the cohort recruited over an eight month period since study initiation.

**Results**

As of 31st July 2014, 106 of 282 screened subjects with CRE were recruited. Sixty-seven (67) of 106 subjects had complete records. Fifty-nine (59) CP-CRE non-duplicate isolates were obtained from these 67 subjects (73.8% CP-CRE of total CRE isolated). More CP-CRE were isolated from surveillance (active surveillance and contact tracing) samples than clinical specimens (64.4% vs. 35.6%; P<0.001). There were significantly more *K. pneumoniae* than *E. coli* (44.1% vs. 22.0%; P<0.001) or any other organism amongst the CP-CRE isolates. 42.4% (25) of the CP-CRE isolates carried the *bla*KPC gene while 35.6% (21) carried the *bla*NDM gene and 16.9% (10) carried the *bla*OXA gene. One isolate, which was a *K. pneumoniae*, expressed both the *bla*NDM and *bla*OXA genes. The distribution of the resistance genes in these isolates is described in Figure 1.

**Conclusions**

Carbapenemase production was the predominant carbapenem resistance mechanism, with *bla*KPC being the most commonly expressed carbapenemase gene in our cohort. The increase in KPC isolates which tend to be clonal, raise concerns of horizontal transmission amongst patients. Tools for appropriate risk stratification of patients are needed to facilitate targeted infection control interventions aimed at curbing further spread of these highly resistant strains within our institutions.
Frequency and Types of Mutations in the rpoB, katG and inhA genes among Mycobacterium tuberculosis Strains in Korea

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Background:

The resistance of Mycobacterium tuberculosis to rifampicin (RIF) and isoniazid (INH) is determined by mutations in the three major genes, rpoG, katG, and inhA. The frequency of mutations related to rpoB, katG and inhA genes in drug-resistant TB strains vary widely around the world. In this study, we investigated the type and frequency of mutations in the rpoB, katG, and inhA genes over a 4-year period obtained from hospitals nationwide.

Methods:

To detect INH- and RIF-resistant M. tuberculosis strains, the katG gene, the regulatory region of the inhA gene, and the 81-bp hot-spot region of the rpoB gene from 554 clinical specimens and 25 culture isolates were sequenced. A total of 579 sequencing results between March 2011 and June 2014 from 61 hospitals nationwide were analyzed.

Results:

One more mutations in the rpoB, katG, and inhA genes were detected in 17.4% of specimens (101 of 579). The point mutations in the hot-spot of rpoB gene were detected in 62 (10.7%) specimens, which were found most frequently at codons 531 (58.1%), 516 (14.5%), 526 (12.9%), 511 (6.5%), 533 (4.8%) and 513 (4.8%) (Table 1). Concurrent mutations in two codons of the rpoB genes were detected in two specimens: codons 511 and 526, 513 and 516, respectively. Of 62 specimens with mutations in rpoB gene, 28 (45.2%) had concurrent mutation in katG gene and 9 (14.5%) had concurrent mutation in inhA gene. Fifty-one (8.8%) specimens had mutations in the codon 315 of the katG gene. The katG gene was shown to bear mutations of two different types: S315T (98.0%), S315N (2.0%). The mutations in the inhA regulatory region were found in twenty-five specimens (4.3%). In the inhA gene, nucleotide substitutions at position -15C>T were most common mutation (84.0%), followed by -8T>C substitution (8.0%).

Conclusions:

This study revealed the frequency and types of mutations in the rpoB, katG, and inhA genes among MTB strains in Korea. These sequencing results could be used for the development of more rapid and simple molecular methods in detection of drug-resistant M. tuberculosis strains in Korea, and would be helpful to diagnosis and control of the drug-resistant M. tuberculosis strains moving among the population.

Keywords: Mycobacterium tuberculosis, inhA, katG, rpoB
Risk Factors for Recurrent *Clostridium difficile* Infection in a University Hospital in Japan

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Background: *Clostridium difficile* infection (CDI) is a highly prevalent healthcare-associated infection. Although most patients respond well to discontinuation of antibiotics and administration of oral metronidazole and vancomycin, 25% of patients experience CDI recurrence. Identification of the risk factors for recurrent CDI may facilitate the early initiation of preventive measures and therapeutic interventions. Despite the increasing recognition of the clinical significance of CDI, very few Japanese studies have used standard surveillance definitions. Here, we aimed to investigate the trend of CDI in a large healthcare facility in Japan, using standard surveillance definitions, and to identify the risk factors for CDI recurrence.

Methods: We conducted a retrospective review of the medical records of healthcare facility-onset CDI patients between August 2011 and September 2013. Patients with diarrhea and positive results for stool CD toxin (detected by an enzyme immunoassay) were diagnosed with CDI. Clinical data, including demographic information, co-morbidities, medications, laboratory parameters, and clinical outcomes, were obtained. We divided the patients into two groups—recurrent and non-recurrent CDI—and investigated the risk factors associated with CDI recurrence.

Results: In total, 79 hospitalized patients were diagnosed with CDI. Of these, 76 patients (96.5%) were categorized as having healthcare facility-onset CDI, with an incidence rate of 0.8 cases per 10000 patient-days. The patients’ median age was 69.5 years (range, 2–95 years), and 50 patients (65.8%) were aged >65 years. Thirty-nine patients (51.3%) were male. Fourteen (18.4%) were recurrent cases, with 13 patients having experienced a single episode and 1 patient having experienced 3 episodes. The 30-day mortality rate was 7.9% (6/76 patients) and the 90-day mortality rate was 14.5% (11/76 patients); however, none of the patient death was related to CDI. A multivariate analysis of risk factors for CDI recurrence showed that the recurrent group was more likely to have underlying malignant disease (odds ratio [OR], 9.08; p=0.03; 95% confidence interval [CI], 1.29–63.94) and a history of hospitalization in an intensive care unit (ICU) (OR, 79.88; p=0.01; 95% CI, 2.56–2488.45).

Conclusions: Compared to reports from the USA and Europe, the proportion of healthcare facility-onset versus community-onset CDI in Japan was higher, but the overall incidence rate was lower.
ICU hospitalization and malignancy were identified as risk factors associated with recurrent CDI. This study is one of only few reports that have investigated the incidence and recurrence of CDI in Japan.
Can contact precautions only decrease the incidence of oxacillin-resistant *Staphylococcus aureus*

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Background  
In medical practice, *Staphylococcus aureus* is one of the most frequent organisms resulting in various infections, which are usually more difficult in treatment compared with that caused by other gram-positive organisms. In addition, the increasing incidence of oxacillin-resistant *S. aureus* (ORSA) also increases difficulty in treatment. The incidence of ORSA is growing in some regions worldwide. Some different interventions have been reported to decrease successfully the incidence of ORSA. In most of hospitals of Taiwan, contact precautions only are implemented as the infection control measure to prevent the spread of ORSA. This study was conducted to explore that whether this measure can decrease the incidence of ORSA.

Methods  
This was a retrospective study at a regional hospital in southern Taiwan. From 2009 to 2013, all *S. aureus* isolates reported from clinical microbiology laboratory were enrolled in this study. According to the susceptibility of oxacillin, *S. aureus* were divided into oxacillin-susceptible *S. aureus* (OSSA) and ORSA in this study.

Results  
A total of 3912 *S. aureus* isolates, including OSSA (*n* = 1736, 44.4%) and ORSA (*n* = 2176, 55.6%), were enrolled. The ORSA accounted for 61.4% (502 of 818), 54.8% (351 of 640), 49.4% (386 of 781), 56% (445 of 795), and 56% (492 of 878) of *S. aureus* in 2009, 2010, 2011, 2012, and 2013, respectively. The incidence of ORSA was statistically significant difference between 2009 and 2011, between 2011 and 2012, and between 2009 and 2012 (*P* < 0.05).

Conclusion  
As a result of this study, from 2009 to 2011, the incidence of ORSA had decreased gradually (61.4% in 2009 versus 49.4% in 2011); however, that increased in 2012 and 2013 (56% in 2012 and 2013). Further studies may be conducted to explore the reasons of increasing incidence of ORSA from 2011 to 2012. On the whole, the incidence of ORSA had decreased from 2009 to 2013 (61.4% in 2009 versus 56% in 2013), indicating that implementing contact precautions only can decrease the incidence of ORSA. However, adding other effective infection control measures to hospital policies may result in more significant decrease in the incidence of ORSA.
The detectable rate of carbapenem-resistant Enterobactericeae by various carbapenems

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Background
Carbapenem-resistant Enterobactericeae (CRE) is defined as Enterobactericeae resistant to any of carbapenems, including ertapenem, imipenem, meropenem, and doripenem. Ertapenem is thought as the most sensitive agent for screening CRE; however, some clinical microbiology laboratories do not test ertapenem. This study was conducted to evaluate the detectable rate of CRE by various carbapenems and combinations.

Methods
This was a retrospective study at a regional hospital in southern Taiwan. From January 2013 to May 2014, all isolates of CRE, defined as the above-mentioned, reported from clinical microbiology laboratory were enrolled in this study. Antimicrobial susceptibility testing was performed by a standard disk diffusion method. The results were interpreted according to criteria recommended by the Clinical and Laboratory Standards Institute. All intermediate results were regarded as resistant in this study.

Results
A total of 80 CRE isolates were collected. The detectable rates of CRE by ertapenem, imipenem, meropenem, doripenem, ertapenem plus imipenem, ertapenem plus meropenem, ertapenem plus doripenem, imipenem plus meropenem, imipenem plus doripenem, and meropenem plus doripenem were 91.3% (n = 73), 47.5% (n = 38), 28.8% (n = 23), 36.3% (n = 29), 100% (n = 80), 91.3% (n = 73), 92.5% (n = 74), 53.4% (n = 43), 56.3% (n = 45), and 40% (n = 32), respectively.

Conclusion
As a result of this study, although ertapenem is regarded as the susceptible carbapenem for detecting CRE, up to 9.7% of CRE was still missed. A combination of ertapenem plus imipenem had 100% of detectable rate of CRE, whereas a combination of imipenem and meropenem had only 53.4% of that. According to these data, we suggest that antimicrobial susceptibility testing for Enterobactericeae should include ertapenem and imipenem in order to detect CRE. Otherwise, CRE may be underestimated.
The difference between central line-related bloodstream infection and catheter-related bloodstream infection

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Background
Central line-associated bloodstream infection (CLABSI) is a surveillance system, defined as a primary bloodstream infection (BSI) in a patient with central venous catheters (CVC) within the 48-hour period prior to the onset of the BSI and the BSI is not related to other infection sources. However, catheter-related bloodstream infection (CRBSI) is a clinical diagnosis, defined as the identification of same organisms from both blood and catheter tip cultures (≥ 15 colony-forming units) without evidence of secondary bacteremia from other infection sources. The definition of CLABSI is more broad than CRBSI, so CRBSI may be overestimated by using the CLABSI surveillance system. This study was conducted to explore the difference between CLABSI and CRBSI.

Methods
This was a retrospective study at a regional hospital in southern Taiwan. From September 2012 to June 2014, of all CLABSI cases, only those with catheter tip culture were enrolled in this study.

Results
Overall, 89 organisms from 80 CLABSI cases and 38 organisms from 38 CRBSI cases were collected and analyzed. In the 80 CLABSI cases, 47.5% (38/80) of those were CRBSI. In the 37 CLABSI cases caused by gram-positive organisms, 51.4% (19/37) of those were CRBSI, including 82.4% (14/17) in S. aureus, 36.4% (4/11) in coagulase-negative staphylococci, 14.3% (1/7) in enterococci, and 0% (0/2) in other gram-positive cocci. In the 43 CLABSI cases caused by gram-negative organisms, 25.6% (11/43) of those were CRBSI, including 66.7% (8/12) in P. aeruginosa, 20% (1/5) in Acinetobacter baumannii, 7.7% (1/13) in Enterobactericeae, and 11.1% (1/9) in other gram-negative bacilli. In the 13 CLABSI cases caused by Candida, 61.5% (8/13) of those were CRBSI.

Conclusions
In this study, we found that only 47.5% of CLABSI cases were CRBSI. For hospitals using CLABSI surveillance system, we suggest that the difference of both should be known in order to prevent overdiagnosis of CRBSI. In addition, we also found that the possibility of CRBSI was high if the offending organisms of CLABSI were S. aureus, P. aeruginosa, and Candida. However, that was low if the offending pathogens of CLABSI were enterococci, A. baumannii, Enterobactericeae, and other infrequent organisms. According to these data, we suggest that CRBSI should be the presumptive diagnosis if the CLABSI cases caused by S. aureus, P. aeruginosa, and Candida. In that case, removal of CVC is highly suggested to both diagnose and treat CRBSI. In contrast, if the CLABSI cases caused by enterococci, A. baumannii, Enterobactericeae, and other infrequent organisms, CRBSI is highly unlikely, and search for other infection sources resulting in bacteremia is suggested.
The knowledge of hand hygiene among military medical students before and after conducting a workshop on hand hygiene

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Background:

Hospital acquired infections are a major problem among hospitalized patients. Nosocomial infections are commonly transmitted by the hands of healthcare workers. The objective of this study was to compare the knowledge of hand hygiene among the preclinical students and the students with clinical exposure at the General Sir John Kotelawala Defence University (KDU), before and after conducting a workshop on hand hygiene.

Method:

A self-administered, pre-tested validated questionnaire from previous publications, based on hand hygiene guidelines laid down by the World Health Organization, was distributed among the medical students at the KDU before and after conducting a workshop on hand hygiene. A workshop was conducted for a total of 112 students and the questionnaire was redistributed among all the students who attended the workshop. Results were analyzed by comparing the knowledge of the WHO guidelines with the knowledge of hand hygiene among the medical students.

Results:

All 177 students participated in the study before conducting the workshop. There were 104 (58.75%) preclinical students and 73 (41.24%) students who had clinical exposure. The percentages of the preclinical students who knew the importance of hand hygiene before having direct contact with patients, after having direct contact with the patients, before clean/aseptic procedures, after contact with blood/body fluid and after contact with patient’s immediate surrounding were 53.84%, 67.30%, 50.96%, 88.46% and 34.61% respectively. The percentages of the students with clinical exposure who knew the importance of the above components of hand hygiene were 49.31%, 63.01%, 87.67, 94.52% and 27.39% respectively. Seventy-four (71.15%) of preclinical students and 56 (76.71%) students with clinical exposure said they need further education in hand hygiene. Of the 112 students who participated in the workshop there were 68(60.71%) preclinical students and 44(39.28%) students with clinical exposure. After attending the workshop the percentages of the preclinical students who knew the importance of hand hygiene before having direct contact with patients, after having direct contact with the patients, before clean/aseptic procedures, after contact with blood/body fluid and after contact with patient’s immediate surrounding were 77.94%, 79.41%, 91.17%, 95.58% and 70.58% respectively. The percentages of the students with clinical exposure who knew the importance of the above components of hand hygiene were 90.91%, 88.63%, 93.18%, 97.72% and 81.81% respectively.

Conclusion:

In general, the knowledge of hand hygiene among the medical students was not satisfactory. A significant improvement of knowledge about hand hygiene among military medical students was observed after conducting a workshop on hand hygiene.
Ceftaroline (CPT) Comparative Activity Against Lower Respiratory Tract Pathogens (LRTI) Collected from Asia-Pacific Region (APR): 2013 Report from the AWARE Surveillance Program

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Background: Pneumonia is a major cause of adult mortality in Asia. Increasing antimicrobial resistance among LRTI pathogens is a serious public health concern in APR. CPT, the active metabolite of CPT fosamil, is a new cephalosporin approved for the treatment of adults with community-acquired pneumonia. This report from the 2013 Assessing Worldwide Antimicrobial Resistance Evaluation (AWARE) surveillance program reviews the comparative in vitro activity of CPT against LRTI pathogens from APR.

Methods: 2,308 LRTI pathogens were collected in 2013 from 35 medical centers: Australia (5), China (11), Japan (4), South Korea (3), Malaysia (3), Philippines (3), Taiwan (3) and Thailand (3). Susceptibility testing was performed by broth microdilution and interpreted according to CLSI guidelines. Enterobacteriaceae were screened for extended-spectrum beta-lactamases (ESBLs) using ceftazidime, aztreonam and cefotaxime MICs <2mg/L.

Results: Percent susceptibility (% S) of major pathogens vs. antimicrobials tested is presented in the table.

\begin{tabular}{|l|c|c|c|c|c|c|c|c|c|c|}
\hline
Organism (n) & CPT & AMP & AMX & CRO & ERY & LVX & Lzd & MXF & PEN & TZP \\
\hline
S. pneumoniae (496) & 99.6 & -- & 75.8 & 76.8 & 36.3 & 95.6 & 100 & 96.6 & 76.8 & -- \\
S. aureus (970) & 67.4 & na & 32.2 & 32.2 & 27.0 & 37.9 & 100 & 37.9 & -- & 32.2 \\
Methicillin-susceptible S. aureus (312) & 100 & na & 100 & 100 & 62.8 & 90.4 & 100 & 90.4 & -- & 100 \\
H. influenzae (60) & 100 & 65 & 96.7 & 100 & na & 98.3 & na & -- & na & 100 \\
K. pneumoniae (243) & 63.0 & 0.8 & 67.9 & -- & -- & 76.6 & -- & -- & -- & 75.7 \\
K. pneumoniae ESBL-negative (155) & 98.7 & 1.3 & 97.4 & -- & -- & 97.4 & -- & -- & -- & 98.7 \\
E. coli (129) & 48.1 & 24.0 & 58.9 & -- & -- & 50.4 & -- & -- & -- & 89.2 \\
E. coli ESBL-negative (69) & 88.4 & 44.9 & 73.9 & -- & -- & 73.9 & -- & -- & -- & 92.8 \\
\hline
\end{tabular}

AMC=amoxicillin-clavulanate, AMP=ampicillin, CRO=ceftiraxone, CPT=ceftaroline, ERY=erythromycin, LVX=levofloxacin, Lzd=linezolid, MXF=moxifloxacin, PEN=penicillin, TZP=piperacillin-tazobactam, na=no breakpoints,-- not tested or not applicable

Conclusions: CPT demonstrated excellent activity against S. pneumoniae. CPT activity against S. aureus was 67.4% (100% of MSSA isolates were susceptible). Activity against H. influenzae was excellent, and comparable to CRO, TZP and LVX. CPT activity against E. coli and K. pneumoniae was similar to other β-lactams and β-lactam/β-lactamase combinations tested, such that activity was diminished by the production of serine β-lactamases.
In Vitro Activity of Ceftaroline (CPT) against Contemporary Staphylococcus aureus Isolates from Asia-Pacific Region (APR): 2013 Report from The AWARE Surveillance Program

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Background: CPT, the active metabolite of ceftaroline fosamil is a new cephalosporin with anti-staphylococcal activity (including methicillin-resistant S. aureus (MRSA)). This report from Assessing Worldwide Antimicrobial Resistance Evaluation (AWARE), a global CPT surveillance program, summarizes the in vitro activity of CPT against S. aureus isolates collected in APR in 2013.

Methods: Susceptibility testing of 2,335 S. aureus isolates (920 methicillin-susceptible S. aureus, 1,415 MRSA) collected in 2013 from 35 hospitals, in Australia, China, Japan, South Korea, Malaysia, Philippines, Taiwan, and Thailand. Isolates were sourced from specimens from patients with SSTI (abscess, burn, carbuncle, cellulitis/erysipelas, decubitus, furuncle, impetiginous lesions, skin ulcers, and wounds) and lower respiratory tract infections (bronchial washing, bronchial alveolar lavage, endotracheal aspirate, sputum). Testing was performed by broth microdilution and MIC results were interpreted according to CLSI performance standards.

Results: Of the 2,335 S. aureus tested 81.8% were CPT susceptible. All MSSA isolates were CPT susceptible (MIC range 0.06-0.5 mg/L, MIC50/90 0.25 mg/L) while 70.0% of MRSA were CPT susceptible (MIC range 0.12-8 mg/L, MIC50/90 1/2 mg/L). The activity of CPT against MRSA isolates is summarized in the table.

<table>
<thead>
<tr>
<th>Region (No. of sites)</th>
<th>N</th>
<th>0.12</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>MIC50/90</th>
<th>%S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia (5)</td>
<td>226</td>
<td>-</td>
<td>18</td>
<td>149</td>
<td>55</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>0.5/1</td>
<td>98.2</td>
</tr>
<tr>
<td>China (11)</td>
<td>389</td>
<td>2</td>
<td>18</td>
<td>79</td>
<td>91</td>
<td>199</td>
<td>-</td>
<td>-</td>
<td>2/2</td>
<td>48.8</td>
</tr>
<tr>
<td>Japan (4)</td>
<td>129</td>
<td>-</td>
<td>5</td>
<td>37</td>
<td>70</td>
<td>17</td>
<td>-</td>
<td>-</td>
<td>1/2</td>
<td>86.8</td>
</tr>
<tr>
<td>South Korea (3)</td>
<td>154</td>
<td>-</td>
<td>2</td>
<td>39</td>
<td>23</td>
<td>86</td>
<td>4</td>
<td>-</td>
<td>2/2</td>
<td>41.6</td>
</tr>
<tr>
<td>Malaysia (3)</td>
<td>45</td>
<td>-</td>
<td>1</td>
<td>13</td>
<td>28</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>1/1</td>
<td>93.3</td>
</tr>
<tr>
<td>Philippines (3)</td>
<td>170</td>
<td>1</td>
<td>3</td>
<td>153</td>
<td>8</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>0.5/0.5</td>
<td>97.1</td>
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<tr>
<td>Taiwan (3)</td>
<td>162</td>
<td>-</td>
<td>12</td>
<td>75</td>
<td>50</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>0.5/2</td>
<td>84.6</td>
</tr>
<tr>
<td>Thailand (3)</td>
<td>140</td>
<td>-</td>
<td>3</td>
<td>8</td>
<td>47</td>
<td>46</td>
<td>27</td>
<td>9</td>
<td>2/4</td>
<td>41.4</td>
</tr>
<tr>
<td>APR (35)</td>
<td>1415</td>
<td>3</td>
<td>62</td>
<td>553</td>
<td>372</td>
<td>385</td>
<td>31</td>
<td>9</td>
<td>1/2</td>
<td>70.0</td>
</tr>
</tbody>
</table>

n = number of sites; N = number of MRSA isolates; %S = percent susceptible
CPT CLSI Breakpoints: S=≤ 1, I=2, R=≥ 4 mg/L

Conclusions: All MSSA isolates were susceptible to CPT. Marked differences in CPT %S across APR was observed against MRSA (%S ranged from 41.4% in Thailand to 98.2% in Australia).
In vitro Comparative Activity of Ceftaroline (CPT) against Streptococcus pneumoniae (SP) from Multiple Specimen Sources in Asia-Pacific Region (APR): 2013 Report from the AWARE Program

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Background: SP remains a significant pathogen isolated from many infectious processes. High prevalence rates of penicillin (PEN) and erythromycin (ERY) resistance among SP from APR countries have been reported, increasing the need for new effective antimicrobials. CPT, the active metabolite of CPT fosamil, is a cephalosporin with activity against Gram-positive and common Gram-negative pathogens. This report from AWARE (Assessing Worldwide Antimicrobial Resistance Evaluation) summarizes the in vitro comparative activity of ceftaroline against SP isolates collected from APR in 2013.

Methods: 35 hospitals in Australia (5), China (11), Japan (4), South Korea (3), Malaysia (3), Philippines (3), Taiwan (3), and Thailand (3) collected 530 SP isolates from multiple specimen sources in 2013. Susceptibility testing was performed by broth microdilution and MIC results were interpreted according to CLSI performance standards.

Results: The activity of CPT and 7 comparator agents against SP are shown in the table.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>All SP (N=530)</th>
<th>PSSP (N=406)</th>
<th>PISP (N=96)</th>
<th>PRSP (N=28)</th>
<th>ERSP (N=335)</th>
<th>CRSP (N=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT</td>
<td>99.6/0.25</td>
<td>100/0.12</td>
<td>100/0.25</td>
<td>92.9/0.5</td>
<td>99.4/0.25</td>
<td>95.2/0.5</td>
</tr>
<tr>
<td>CRO</td>
<td>76.6/2</td>
<td>93.6/1</td>
<td>27.1/4</td>
<td>0/&gt;4</td>
<td>63.9/4</td>
<td>0/&gt;4</td>
</tr>
<tr>
<td>CLI</td>
<td>46.4/&gt;1</td>
<td>55.2/&gt;1</td>
<td>21.9/&gt;1</td>
<td>3.6/&gt;1</td>
<td>16.1/&gt;1</td>
<td>2.4/&gt;1</td>
</tr>
<tr>
<td>ERY</td>
<td>36.2/&gt;1</td>
<td>46.3/&gt;1</td>
<td>4.2/&gt;1</td>
<td>0/&gt;1</td>
<td>0/&gt;1</td>
<td>0/&gt;1</td>
</tr>
<tr>
<td>LVX</td>
<td>95.9/2</td>
<td>97.8/2</td>
<td>92.7/2</td>
<td>78.6/&gt;8</td>
<td>93.4/2</td>
<td>81.0/&gt;8</td>
</tr>
<tr>
<td>LZD</td>
<td>100/2</td>
<td>100/2</td>
<td>100/1</td>
<td>100/1</td>
<td>100/1</td>
<td>100/1</td>
</tr>
<tr>
<td>MXF</td>
<td>96.8/0.12</td>
<td>98.0/0.12</td>
<td>96.9/0.25</td>
<td>78.6/4</td>
<td>94.9/0.25</td>
<td>83.3/4</td>
</tr>
<tr>
<td>PEN</td>
<td>76.6/4</td>
<td>100/2</td>
<td>0/4</td>
<td>0/&gt;8</td>
<td>64.5/4</td>
<td>2.4/8</td>
</tr>
</tbody>
</table>


ALL SP: All SP collected, PSSP: PEN-susceptible SP (PEN MIC ≤2 mg/L), PISP: PEN-intermediate SP (PEN MIC=4 mg/L), PRSP: PEN-resistant SP (penicillin MIC ≥8 mg/L), ERSP: ERY-resistant SP (ERY MIC ≥1 mg/L), CRSP: CRO-resistant SP (CRO MIC ≥4 mg/L).

Conclusions: Among the 530 SP isolates collected, 23.4% were non-susceptible to PEN and 63.8% were resistant to ERY. CPT demonstrated potent (≥99%) in vitro activity against PSSP, PISP, and ERSP isolates. CPT activity was only slightly lower (92.9%, and 95.2%) against PRSP and CRSP isolates, respectively. Overall, the susceptibility of SP to CPT was comparable to LVX and MXF at their respective CLSI breakpoints for all SP, PSSP, PISP and ERSP while CPT demonstrated enhanced activity against PRSP and CRSP in comparison to LVX and MXF.
Susceptibility and Multi-Drug Resistance among *E. coli* from Intra-Abdominal Infections in Asia/Pacific – SMART 2012-2013
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IHMA, Inc., Schaumburg, IL, USA

**Background:** *Escherichia coli* is one of the most common causes of many infectious diseases, including intra-abdominal infections (IAI). Although this species has largely remained susceptible to many antimicrobics, increasing resistance has been reported to several classes of drugs, and has reached alarming levels in some parts of the world. This report from the Study for Monitoring Antimicrobial Resistance Trends (SMART) analyzes the susceptibility of *E. coli* including rates of multi-drug resistant (MDR) isolates in Asia/Pacific in 2012 and 2013.

**Methods:** 4,186 UTI *E. coli* were collected at 65 sites in 13 Asia/Pacific countries and Hong Kong in 2012-2013. Susceptibility was determined using the CLSI broth microdilution method and breakpoints. MDR isolates were defined as resistant to three or more antimicrobial drug classes (aminoglycosides, β-lactam/β-lactamase inhibitor, cephems, carbapenems, and quinolones). An IAI was deemed hospital-associated or community-associated if cultured ≥48 hours or <48 hours from admission, respectively.

**Results:** MDR rates, ESBL rates, and % susceptible of *E. coli* selected agents are shown below by country (in descending order of MDR rates) and for Asia/Pacific overall; % susceptible values ≥90% are shaded:

<table>
<thead>
<tr>
<th>Country (# sites)</th>
<th>n</th>
<th>% MDR</th>
<th>% ESBL</th>
<th>AMK</th>
<th>FEP</th>
<th>CTX</th>
<th>CAZ</th>
<th>ETP</th>
<th>IPM</th>
<th>LVX</th>
<th>SAM</th>
<th>TZP</th>
</tr>
</thead>
<tbody>
<tr>
<td>India (6)*</td>
<td>97</td>
<td>61.9</td>
<td>72.2</td>
<td>85</td>
<td>33</td>
<td>27</td>
<td>28</td>
<td>79</td>
<td>89</td>
<td>30</td>
<td>25</td>
<td>67</td>
</tr>
<tr>
<td>China (21)</td>
<td>1618</td>
<td>47.4</td>
<td>67.3</td>
<td>93</td>
<td>43</td>
<td>32</td>
<td>55</td>
<td>93</td>
<td>97</td>
<td>33</td>
<td>18</td>
<td>89</td>
</tr>
<tr>
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<td>97</td>
<td>23.7</td>
<td>49.5</td>
<td>99</td>
<td>51</td>
<td>44</td>
<td>52</td>
<td>99</td>
<td>99</td>
<td>41</td>
<td>22</td>
<td>96</td>
</tr>
<tr>
<td>Vietnam (4)*</td>
<td>240</td>
<td>22.9</td>
<td>46.7</td>
<td>97</td>
<td>53</td>
<td>45</td>
<td>58</td>
<td>98</td>
<td>98</td>
<td>57</td>
<td>30</td>
<td>90</td>
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<td>19.1</td>
<td>19.1</td>
<td>99</td>
<td>82</td>
<td>66</td>
<td>70</td>
<td>99</td>
<td>98</td>
<td>55</td>
<td>29</td>
<td>94</td>
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<td>30.3</td>
<td>99</td>
<td>75</td>
<td>69</td>
<td>82</td>
<td>100</td>
<td>100</td>
<td>60</td>
<td>35</td>
<td>91</td>
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<td>100</td>
<td>81</td>
<td>74</td>
<td>83</td>
<td>99</td>
<td>100</td>
<td>52</td>
<td>45</td>
<td>95</td>
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<td>100</td>
<td>100</td>
<td>64</td>
<td>52</td>
<td>94</td>
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<td>Taiwan (8)</td>
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<td>15.3</td>
<td>99.5</td>
<td>86</td>
<td>68</td>
<td>71</td>
<td>99</td>
<td>99</td>
<td>73</td>
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<td>93</td>
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<td>100</td>
<td>86</td>
<td>78</td>
<td>84</td>
<td>98</td>
<td>100</td>
<td>79</td>
<td>52</td>
<td>96</td>
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<td>Japan (3)</td>
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<td>19.4</td>
<td>100</td>
<td>87</td>
<td>72</td>
<td>79</td>
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<td>100</td>
<td>51</td>
<td>58</td>
<td>97</td>
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<td>100</td>
<td>87</td>
<td>72</td>
<td>84</td>
<td>99</td>
<td>100</td>
<td>75</td>
<td>36</td>
<td>96</td>
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<td>11.1</td>
<td>100</td>
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<td>87</td>
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<td>100</td>
<td>99</td>
<td>88</td>
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<td>89</td>
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<td>96</td>
<td>65</td>
<td>54</td>
<td>67</td>
<td>96</td>
<td>98</td>
<td>55</td>
<td>32</td>
<td>92</td>
</tr>
</tbody>
</table>

* Only data for 2012 available.

AMK=amikacin, FEP=cefepime, CTX=cefotaxime, CAZ=ceftazidime, ETP=ertapenem, IPM=imipenem, LVX=levofloxacin, SAM=ampicillin-sulbactam, TZP=piperacillin-tazobactam.

The MDR rate in Asia/Pacific overall was significantly higher in hospital-associated (32.7%) than community-associated IAI (18.1%, p<0.0001, chi-square test).

**Conclusions:**
- MDR rates for IAIE, coli in Asia/Pacific varied widely by country, ranging from 3% in Australia to 62% in India. ESBL rates showed a similar pattern.
- AMK, ETP, and IPM were the most active agents, inhibiting ≥97% of *E. coli* from IAI in all countries except India and China. In India, none of the tested agents inhibited ≥90% of isolates.
- Carbapenem activity remains high in most countries in Asia/Pacific, but lower susceptibility in China and especially India corroborates reports of increases in carbapenemases in these countries.
- Multi-drug resistance seriously limits therapeutic options in many countries in the Asia/Pacific region. Monitoring the susceptibility of *E. coli* at the regional, country, and even hospital level is crucial to help guide antibiotic therapy.
**Carbapenem-/Multidrug-Resistant Enterobacteriaceae (CRE/MDR) Rates, and the Distribution of ESBL-, and Carbapenemase-Producing Isolates in Ten Asia-Pacific Countries (SMART Program 2013)**

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**Background:** The emergence, spread and increasing prevalence of carbapenem resistant Enterobacteriaceae (CRE) has resulted in difficult to treat infections. Carbapenemase producers have been documented in all geographic regions with variability of enzymes detected and endemic occurrences observed in some countries. This study documents the rate and distribution of MDR Enterobacteriaceae, and the types of ESBLs and carbapenemases in Asia-Pacific (AP) countries.

**Methods:** Hospitals in ten AP countries contributed 3,556 Enterobacteriaceae isolates in 2013 as part of the global SMART program. Susceptibility (S) testing was performed by broth microdilution and MIC results were interpreted according to CLSI performance standards. β-lactamase genes were detected and analyzed by PCR and sequencing for all CRE and a randomly selected 50% of phenotypically ESBL-positive isolates.

**Results:**

<table>
<thead>
<tr>
<th>Country/(# isolates)</th>
<th>MDR (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CRE (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Carbapenemase genes detected</th>
<th>ESBL genes detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia (512)</td>
<td>3.9</td>
<td>1.7/0.8</td>
<td>None</td>
<td>SHV-12; CTX-M-14,15,27,79</td>
</tr>
<tr>
<td>Hong Kong (257)</td>
<td>16.7</td>
<td>0.9/1.7</td>
<td>None</td>
<td>CTX-M-3,14,15,27,55</td>
</tr>
<tr>
<td>Japan (242)</td>
<td>11.2</td>
<td>3.5/5.0</td>
<td>IMP-1; IMP-6</td>
<td>SHV-12; CTX-M-2,14,15</td>
</tr>
<tr>
<td>Korea, South (255)</td>
<td>17.3</td>
<td>2.9/0.0</td>
<td>None</td>
<td>SHV-12; CTX-M-14,15,24,27,55</td>
</tr>
<tr>
<td>Malaysia (240)</td>
<td>12.5</td>
<td>0.5/0.9</td>
<td>NDM-1</td>
<td>CTX-M-14,15,27,55,65</td>
</tr>
<tr>
<td>New Zealand (426)</td>
<td>6.1</td>
<td>1.0/1.0</td>
<td>None</td>
<td>CTX-M-14,15,27</td>
</tr>
<tr>
<td>Philippines (283)</td>
<td>23.0</td>
<td>6.6/6.6</td>
<td>NDM-1; NDM-7; IMP-26</td>
<td>SHV-12; CTX-M-14,15,26,27,55</td>
</tr>
<tr>
<td>Singapore (263)</td>
<td>17.1</td>
<td>3.2/0.0</td>
<td>None</td>
<td>SHV-2a,12; CTX-M-14,15,27,65</td>
</tr>
<tr>
<td>Taiwan (978)</td>
<td>11.7</td>
<td>5.6/2.5</td>
<td>VIM-1</td>
<td>SHV-2,12,31; CTX-M-3,14,15,24,27,55,65,79</td>
</tr>
<tr>
<td>Thailand (100)</td>
<td>23.0</td>
<td>4.0/3.0</td>
<td>NDM-1</td>
<td>CTX-M-14,15,24,27,55,63</td>
</tr>
</tbody>
</table>

<sup>a</sup> MDR, resistant to ≥3 drug classes; 3<sup>rd</sup> and/or 4<sup>th</sup>-generation cephalosporins, piperacillin/tazobactam, carbapenems, fluoroquinolones, amikacin.

<sup>b</sup> Non-susceptible to ertapenem/imipenem, excludes Proteae.

**Conclusions:** Variations in MDR and CRE rates were observed in AP countries. CTX-M-type β-lactamases were the most common ESBL enzymes observed in this region. KPC and OXA-48 carbapenemases were not observed in this AP isolate sample, but NDM, IMP, and more rarely, VIM, were found in several countries.
Antimicrobial susceptibility patterns among gram negative bacteria in Center of Pathology Diagnostic and Research (CPDRL) UiTM Sungai Buloh. NSahan, F R MohdRustam, N A Mohd Shah, F MohdNor Ghazali, N H Ramli

Center of pathology diagnostic and research Laboratories, University Teknologi MARA, Malaysia

Introduction: Resistance rate among gram negative bacteria is increasing at an alarming rate. Important causes of resistance among gram negative bacteria is Extended spectrum beta lactamase enzyme (ESBL). Studies have shown that emergence of ESBL producing organisms lead to increase morbidity and mortality. In providing empirical therapy, it is important to consider the local prevalent resistance patterns. Thus, a local resistance pattern at CPDRL UiTM Sungai Buloh is needed to provide data that can be used as a guide for appropriate antimicrobial therapy.

Method: A retrospective study was conducted. The gram negative isolates cultured at Centre of Pathology Diagnostic and Research Laboratories (CPDRL) of UiTM Sungai Buloh from January 2011 to June 2014 were identified. The susceptibility patterns for each isolates were determined by reviewing their antimicrobial susceptibility test results.

Results: In the period of 42 months, there were 140 gram negative isolates cultured. 14 bacterial species were identified; Klebsiella pneumoniae was the most common isolate (22%), followed by Escherichia coli (20%) and Pseudomonas aeruginosa (18.6%). Majority of E. coli is resistant to ampicillin (73.7%) but remain sensitive to ciprofloxacin (85.7%), ceftaxime (71.4%) and piperacillin-tazobactam (100%). Majority of K pneumonia are sensitive to ciprofloxacin (80.6%), piperacillin-tazobactam (80%) cotrimoxazole (80.6%), amoxicillin-clavulanate (61.3%) and cefazidime (96.2%). Majority of P aeruginosa are sensitive to piperacillin-tazobactam (100%), gentamicin (92.3%), ciprofloxacin (96.2%) and ceftazidime (96.2). 100% of Salmonella sp are sensitive to ciprofloxacin and 80% are sensitive to ceftriaxone. Majority of Acinetobacter sp are sensitive to ampicillin-sulbactam (75%). 24.2% of screened organisms were ESBL positive and majority of them were E. coli (50.0%), followed by K. pneumoniae (31.3%) and Proteus mirabilis (18.8%). 42.9% of Proteus mirabilis, 28.6% of E coli and 16.1% of K pneumonia were ESBL producing.

Conclusions: The above findings provide a much needed local data on the prevalence of gram negative isolates and their antimicrobial susceptibility patterns at the Centre of Pathology Diagnostic and Research Laboratories (CPDRL) of UiTM Sungai Buloh.
Co-existence of NDM-1 with OXA-48 like enzymes in Gram negative organisms in Islamabad, Pakistan

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Introduction: Since its discovery in 2009, New Delhi metallo-β-lactamase (NDM) enzymes have been reported in many countries around the globe. OXA-48-type carbapenemases have also been increasingly reported in enterobacterial species. The enzymes hydrolyse carbapenems at a low level, but when combined with permeability defects, OXA-48-like producers may exhibit a high level of resistance to carbapenems. The co-existence of NDM-1 with other beta-lactamases makes these strains very difficult to treat. Pakistan is one of the countries in South Asia where high carriage of NDM-1 has been reported and also faces the challenges of multi-drug resistant (MDR) bacteria. The objective of current study was to determine the co-existence of blaNDM-1 with other beta-lactamase enzymes in Gram negative organisms.

Methods: Gram negative bacteria were isolated from patients coming to a tertiary care hospital in Islamabad, Pakistan. Antibiotic susceptibility profiling was performed by disk diffusion method and MICs for colistin and tigecycline were performed using E-test strips. Isolates were screened for the presence of blaNDM-1 by PCR which was confirmed by Southern blotting. PFGE was performed to analyze the chromosomal/plasmid carriage of this gene. Strains were further analyzed for the presence of OXA-48 variants, IMP, VIM-2, AmpC-β-lactamase and CTX-M by PCR amplification.

Results: A total of 323 Gram negative rods were collected from clinical samples of which 85 were found positive for blaNDM-1 by PCR. Out of these 85 isolates, 18 were E.coli, 22 were K.pneumoniae, 15 were Pseudomonas spp., 12 were Acinetobacter spp., 12 were Enterobacter cloacae, 6 were Providencia rettgeri and one isolate was Morganella morganii. PFGE analysis showed that blaNDM-1 was found both on chromosomes and plasmids of sizes ranging from 150kb to 700kb. Four strains of Pseudomonas spp. were positive for blaVIM-2. Of blaNDM-1 positive isolates, 39 (46%) showed co-existence of OXA-48 variants. Thirty four isolates were positive for CTX-M genes belonging to group I. AmpC from CTM family were identified in 16 isolates. None of the NDM-1 positive strains showed the presence of IMP beta-lactamases. All blaNDM-1 positive isolates were MDR showing resistance to major antibiotic groups with MICs for tigecycline ranging from 0.19 to 8µg/mL and for colistin from 0.25 to 2µg/mL.

Conclusion: OXA-48 like enzymes has not been reported previously in isolates from Pakistan. Their high prevalence and co-existence with NDM-1 and other beta-lactamases is a point of concern and should be further investigated.
Susceptibility Pattern and Associated Risks in Patients with Coagulase negative *Staphylococci*

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**Background**: Coagulase Negative *Staphylococcus* or CoNS is a gram positive bacteria that has been acknowledged as possible skin and blood contaminant. Its ability to produce slime or biofilm makes this microorganism more virulent and causes life-threatening infections especially among the hospitalised patients. There are difficulties in determining the significance of these isolate unless further molecular testing is done to detect the specific virulent genes. As clinical microbiologist we are in dilemma to interpret the significance of the CoNS results without relevant clinical history. There is a need to know which ward has frequent CoNS isolated so that further investigation can be done by Infection Control Team to determine the significance of these isolates. This study was conducted to determine the distribution of CoNS in different departments, local susceptibility pattern and describe the associated risks in patients with coagulase negative *Staphylococcus* species in Hospital Serdang.

**Methods**: A hospital-based retrospective descriptive study was conducted by assessing secondary data from 2012 to 2013 out at the Department of Pathology, Hospital Serdang. A number of 246 patients were selected by systematic random sampling according to criteria. These data were accessed from the hospital information system after getting approval from Clinical Research Centre and Medical Research and Ethics Committee, Ministry of Health. The data recorded were demographic factors, associated risks, underlying medical conditions, laboratory report and antimicrobial susceptibility results. All data were recorded in standardized pro forma.

**Results**: The medical ward was recorded the highest percentage of CoNS (28.5%) followed by emergency department and nephrology ward with 16.7 % and 11.8% respectively. Most of the patients with CoNS were adults (53.7%), diabetic (56.9%) and had underlying sepsicaemia (39.8%). Majority (94.3%) of CoNS were isolated from blood specimens. It was reported that CoNS has the highest sensitivity towards vancomycin (99.1%), whereas the highest resistance was towards Penicillin G (80.9%). Most of the patients were found to have peripheral line (85.4%) followed by the use of other catheters (45.9%).

**Conclusion**: Majority of patients with CoNS were from the medical ward. It was postulated that there was more usage of peripheral lines and catheter in the medical ward contributing to the highest percentage of CoNS in this ward. However, the significance of these microorganisms needs to be further investigated and correlated with clinical findings to determine true infection or colonisation.
Nasal Colonization of Pathogenic Bacteria Among Medical Students

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**Background:** The normal flora members have different numbers and types for each site in the body. They are the permanent residents in the body and somehow, they also become the colonizer. After colonizing part of the human body, they either cause infections or may be eliminated by our host defense. Nose or anterior nares is considered a place for bacterial reservoir that harbour potentially pathogenic bacteria like *Staphylococcus aureus, Streptococcus pneumoniae, Moraxella catarrhalis, Neisseria meningitidis, Haemophilus influenzae* and other gram negative bacteria like *Escherichia coli, Klebsiella pneumoniae* and *Enterobacter* spp. These bacteria colonize and reside at the nasal passages without causing harm to human population and therefore considered commensal. However, these bacteria are also responsible for a great deal of morbidity and mortality. This study was conducted to determine the prevalence of nasal colonization of bacterial pathogens, identify the distribution of different bacterial pathogens isolated from anterior nares of medical students and to compare the colonization rate of different bacterial pathogens between preclinical and clinical medical students in Faculty of Medicine and Health Sciences (FMHS), UPM.

**Methods:** A cross-sectional study was conducted on 100 medical students in FMHS, UPM. The nasal swabs were collected and cultured onto chocolate agar and 5% sheep-blood agar. Each colony type was sub-cultured onto chocolate agar, 5% sheep-blood agar and Mac-Conkey agar. Identification of the pathogens are based on colonial morphology on growth & differential media, Gram stain microscopy and biochemical tests such as oxidase, catalase, coagulase, sugar fermentation tests and semi-automated Analytical Profile Index (API) system.

**Results:** Of 100 samples, 41(41%) pathogenic bacteria were isolated from anterior nares. They were *Staphylococcus aureus* (39%), *Klebsiella pneumoniae* (26.8%), *Enterobacter* spp (12.2%), *Moraxella catarrhalis* (7.6%), *Pseudomonas stutzeri* (7.6%) and *Escherichia coli* (7.6%). It was reported that the clinical students with 27 positive pathogenic isolates (27%) have higher nasal colonization rate than preclinical students with 14 positive pathogenic isolates (14%). This study also found the significant association between clinical exposure and nasal colonization of pathogenic bacteria among UPM medical students (p<0.05).

**Conclusion:** The prevalence of nasal colonization of bacterial pathogens in this study is 41% in which *Staphylococcus aureus* is the most predominant pathogen. The clinical students have higher nasal colonization rate than preclinical students that have no clinical exposure. It was probably due to more exposure to hospital environment among the clinical students. There is significant association between clinical exposure and nasal colonization of pathogenic bacteria among medical students in UPM.
CHARACTERIZATION OF GROUP B STREPTOCOCCUS ISOLATED FROM THREE MAJOR HOSPITALS IN MALAYSIA

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Background: Group B Streptococcus (GBS) or also known as Streptococcus agalactiae is the frequent colonizer in pregnant women’s vagina and also gastrointestinal and genitourinary tract of healthy adults. GBS can cause sepsis and meningitis in neonates. It also promotes serious mortality in immunocompromised patients and morbidity in pregnant women and nonpregnant adults. Capsular polysaccharide (CPS) is assumed as primary virulence factors in GBS pathogenicity. There are ten serotypes (Ia, Ib, II until IX) based on their unique CPS antigens. Serotype Ia, III and V are mostly present in the infection of pregnant women and neonates while type V is the common serotype in nonpregnant adults. This study examined the distribution of serotypes of sixty GBS isolates and their potential genetic relation in three major hospitals serving the densely populated area of Klang Valley.

Methods: All GBS isolates were obtained from different patients admitting to Hospital Serdang, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) and Hospital Kuala Lumpur from December 2012 until March 2014. Ten isolates from each sterile and non-sterile site were randomly collected from each hospital. Serotyping was performed by latex agglutination method using specific antisera. The process was confirmed further by multiplex PCR. The genetic relation between the isolates was examined by Random Amplified of Polymorphic DNA (RAPD) based typing using P2 and GBS2 primer. A composite dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method.

Results: Serotype Ia was the most common, (n = 27, 45 %) followed by serotype III (n =10, 16.7 %), V (n = 9, 15 %), VI (n = 8, 13.3 %), II (n = 3; 5 %) VIII and VII (n = 2; n = 1; 3.3 %, 1.7 %). Serotype Ib, IV and IX were not found. Based on RAPD, the study showed a diverse genetic pedigree of all isolates in four major clusters. At 31% of similarity, isolates were segregated in the dendrogram according to their isolation sites and location of hospitals. There were no obvious genetic linkages in relation to serotypes.

Conclusion: This study preliminary suggest the occurrence of distinct GBS with serotype Ia being the most common regardless of the isolation site and location.
The Pharmacodynamics Optimization of Intermittent Vancomycin Dosage Regimens in Methicillin-Resistant Staphylococcus aureus Infections with MIC of 1.5 and 2.0 mg/L in Thai Population

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2Faculty of Pharmacy, University of Surabaya, East Java, Indonesia.

Keywords: Vancomycin, Methicillin-Resistant Staphylococcus aureus, Pharmacodynamics

Background
There are increasing number of articles questioning the efficacy of vancomycin to treat methicillin-resistant Staphylococcus aureus (MRSA) with MIC 1.5mg/L and 2.0mg/L. However, vancomycin is still used as the cornerstone treatment of MRSA infection particularly in most of developing countries with limited alternative MRSA coverage antibiotics. Owing to the interest whether vancomycin still enable to be used as the cornerstone treatment for MIC 1.5mg/L and 2mg/L, present study was conducted to analyze the achievement of vancomycin desired PK-PD indices in MRSA-infected Thai population.

Methods
Monte Carlo simulation by using 10,000 replications was performed for several vancomycin intermittent dosage regimens ranging from 1g every 6, 8, 12h, 1.5g and 2 g every 12h. Vancomycin concentrations were estimated from population PK study conducted in 212 Thai population. The probability of target attainment (PTA) of each intermittent dosage regimen was calculated from the number of simulated patients who achieved AUC24/MIC ≥400 for MIC 1.5mg/L and 2.0mg/L divided by total number of replication.

Results
Dosage regimen 1g every 12h couldn’t afford desired PTA for MRSA with MIC 1.5mg/L and 2.0mg/L. Considering the MRSA with MIC 1.5mg/L, dosage regimen 1g every 8h and 1.5g every 12h could afford PTA >80%. However, if particular conditions required PTA >90%, dosage regimen 1g every 6 hours or 2g every 12h should be recommended as the most appropriate dosage regimen. While, for MRSA with MIC 2.0mg/L, only dosage regimens 4g/day, either given as 1g every 6h or 2g every 12h, could afford PTA >80%. No any dosage regimens could afford PTA >90% for MRSA with MIC 2.0mg/L. All PTA achievement represented the PTA at steady state condition.

Conclusions
Intermittent dosage regimen at least 3g/day and 4g/day were needed to afford desired PTA achievement for MRSA with MIC 1.5mg/L and 2.0mg/L, respectively. Finding of present study could be used as a guidance in determining the best intermittent dosage regimen in documented vancomycin treatment. Further study was needed to determine the most appropriate intermittent dosage regimen that could achieve the desired PTA for the 1st 24h.
THE EFFECTS OF ETHANOL EXTRACT AND ESSENTIAL OIL OF RED BETEL VINE (*Piper crocatum*) LEAVE TOWARDS CELL WALLS OF *Mycobacterium tuberculosis*

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**ABSTRACT**

**Research Background:** Red betel vine (*Piper crocatum*) plant is one of Indonesia's natural wealth that can serve as a drug. Rachmawaty (2012) have reported that the ethanol extract and essential oil of red betel vine is proven to have antimycobacterium activity against *Mycobacterium tuberculosis* (*M. tuberculosis*). Thus, this study aims to identify the effect of ethanol extract and essential oil of red betel vine leave towards the cell wall of *M. tuberculosis*.

**Methods:** This research is anlaboratory experimental research. Red betel vine leaves was extracted using maceration method with ethanol solvent. Meanwhile, the essential oil was isolated by distillation according to the standard method. Ethanol extracts and essential oils were exposed in *M. tuberculosis* in forty eight hours (2 days) on microplate. *M. tuberculosis* is then examined under the electron microscope according to the right procedure. This study also included controls of *M. tuberculosis* without exposure and controls with exposure to isoniazid.

**Results:** The findings showed that the cell wall of *M. tuberculosis* exposed to ethanol extracts had damage. Compared to *M. tuberculosis* without exposure, it can be observed that the cell wall of *M. tuberculosis* exposed to ethanol extract and essential oil became not intact. This also happened on the cell wall of *M. tuberculosis* exposed to isoniazid. Damage that occurs on essential oil exposure is more severe than the exposure to ethanol extract.

**Conclusion:** Ethanol extract and essential oil of red betel vine leave causes damage to the cell wall of *M. tuberculosis*. Consequently, damage that occurs in the cell wall kill *M. tuberculosis*.

**Key words:** ethanol extracts, essential oils, red betel vine (*Piper crocatum*), *M. tuberculosis*
Abstract Title: Chikungunya virus: An Australian exploration of the imminent global threat

Abstract ID: 356 (Emerging infections)

Authors: KEEM Michael, MASON Emma, CHEUNG King Tung, SHARP Lauren, LING Rebecca, CHENG Yik Sheng, LI Simon, LO Giselle, SRISKANDAN Sujinyaa, Sun Ying Sunny, TEDJASEPUTRA Aditya, KUK Nathan

INTRODUCTION: Chikungunya virus (CHIKV), an ‘old world’ alphavirus transmitted by mosquitoes, has drawn significant global research attention in recent years. It has led to extensive morbidity since re-emerging in 2004 with a number of explosive and unpredictable outbreaks, and is poised to become endemic in Australia. While CHIKV was first described over 60 years ago, it remains poorly understood relative to other arboviruses such as dengue. Here, we explore the reasons for the alarming increase in CHIKV cases reported in Australia and the substantial threat it poses.

METHODS: Cases reported to the National Notifiable Disease Surveillance System from 2008-2013 were analysed by country of acquisition. We correlated these findings to data from the Australian Bureau of Statistics on short-term movements of travellers from 6 countries identified as key global, Oceanic or Southeast Asian sources of CHIKV to estimate rates of CHIKV importation. We furthermore undertook a comprehensive literature review using the keywords ‘chikungunya’, ‘CHIK’, ‘CHIKV’ and ‘CHIKF’ on PubMed and Discovery (University of Melbourne).

RESULTS: While the number of CHIKV cases was largest from Indonesian, Indian and Malaysian short-term movements, the relative risk of infection was significantly higher from East Timor. Short-term movements to Bali underpin the considerable spike in CHIKV cases in 2013. Virus mutation, expanding geographical range of vectors, low pre-travel medical consultation rates, poor use of vector prophylaxis, global warming and inadequate targeting of the key patterns of arbovirus spread have all contributed to the increased numbers of CHIKV infections in the countries analysed.
CONCLUSION: Chikungunya is an emerging infection in Australia and continues to be a serious concern worldwide. Currently, travel is entirely accountable for Australian notifications. These can be curbed with public health measures, continued case and vector surveillance, targeting of key travel demographics, more efficient and targeted use of vector prophylaxis, continued vaccine development, and post-exposure immunoglobulin prophylaxis.
Ertapenem in outpatient parenteral antibiotic therapy for complicated urinary tract infections

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Abstract:

Background: Management of complicated urinary tract infections (cUTIs) has long been based on fluoroquinolone therapy, but emerging resistance patterns to antimicrobials have rendered them less effective. Ertapenem has been demonstrated to be efficacious in treating cUTIs. It is increasingly being utilized against cUTIs due to Multi-Drug Resistant Organisms (MDROs) in the outpatient setting. This study describes the clinical response and microbiologic cure rates associated with ertapenem treatment for cUTIs in two Outpatient Parenteral Antibiotic Therapy (OPAT) departments in Singapore.

Methods: A multi-centre, open-labeled, prospective, and observational study of patients who received ertapenem for cUTIs between August 2010 and August 2014 in two major Singaporean OPAT departments. Patient characteristics, microbiological causes as well as antibacterial susceptibilities, clinical course of infection, adverse events, and outcomes of therapy in patients were analysed. Microbiologic response was defined as a negative culture on any occasion after treatment. Clinical response was measured as total resolution of signs and symptoms.

Results: 61 patients were enrolled and received ertapenem therapy in OPAT during the study period. The mean age was 59 years (range 24, 83) and 59% were male. The most common causative organism was Escherichia coli (62%). ESBL organisms were detected in 53% of infections. The most common type of infection included pyelonephritis (39%), UTI (39%), prostatitis (16%), and UTI with bacteraemia (3%). Duration of therapy lasted an average of 12 days (range: 3, 38). Out of the 61 patients, 3 (5%) experienced an adverse reaction. Clinical response was observed in 92% of patients. Microbiologic cure was achieved in 68% of the evaluable population (n = 56).

Conclusion: In this study of treating cUTIs caused by MDROs with ertapenem, we have demonstrated low toxicity (5%) and good clinical recovery (92%). A lower than expected microbiologic cure rate suggests a possible need for prolonged courses compared with those with non MDRO infections.
Molecular profiling of multidrug resistant *Enterobacteriaceae* collected globally as part of the Tigecycline Evaluation and Surveillance Trial (2005-2011)

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²Pfizer Inc., Collegeville, PA, USA
³IHMA Europe Sàrl, Epalinges, Switzerland

**Background**: The Tigecycline Evaluation Surveillance Trial (TEST) monitors activity of tigecycline (TGC), a glycylcycline approved for treatment of complicated skin and skin structure and intra-abdominal infections, and comparators against clinically-relevant pathogens collected globally. Multidrug resistant (MDR) isolates collected from the TEST program were molecularly characterized for \(\beta\)-lactamase (bla) genes and examined for regional differences in antibiotic susceptibility patterns.

**Methods**: Susceptibility testing of isolates was performed using broth microdilution (CLSI M100-S24). 1980 MDR Enterobacteriaceae, defined as resistant to three or more antimicrobials, were selected from isolates collected through the TEST global program from 2005 to 2011, and 1257 of these (103 from Asia/Pacific, 551 from Europe, 77 from Middle East/Africa, 328 from Latin America, and 198 from North America) were molecularly characterized using a combination of multiplex PCR and microarray. Genes encoding extended-spectrum \(\beta\)-lactamases (ESBL) or carbapenemases were sequenced.

**Results**:

<table>
<thead>
<tr>
<th>Enzyme group (n)</th>
<th>TGC</th>
<th>IMI/MEM</th>
<th>FEP</th>
<th>CAZ</th>
<th>TZP</th>
<th>AMK</th>
<th>LVX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asia/ Pacific</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (103)</td>
<td>2</td>
<td>0.5</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
<td>&gt;64</td>
<td>&gt;8</td>
</tr>
<tr>
<td>ESBL (83)</td>
<td>2</td>
<td>0.5</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
<td>&gt;64</td>
<td>&gt;8</td>
</tr>
<tr>
<td>AmpC (2)</td>
<td>0.25-1</td>
<td>0.12-1</td>
<td>&lt;0.5-8</td>
<td>8-&gt;32</td>
<td>2-16</td>
<td>16</td>
<td>1-&gt;8</td>
</tr>
<tr>
<td>ESBL+AmpC (13)</td>
<td>4</td>
<td>0.5</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
<td>&gt;64</td>
<td>&gt;8</td>
</tr>
<tr>
<td>SerCp’ase+ESBL (3)</td>
<td>0.25-1</td>
<td>0.12-16</td>
<td>16-&gt;32</td>
<td>8-&gt;32</td>
<td>32-128</td>
<td>2-16</td>
<td>2-&gt;8</td>
</tr>
<tr>
<td>MBL+ESBL (1)</td>
<td>1</td>
<td>4</td>
<td>32</td>
<td>&gt;32</td>
<td>&gt;128</td>
<td>&gt;64</td>
<td>0.12</td>
</tr>
<tr>
<td>No bla gene detected (1)</td>
<td>1</td>
<td>&gt;16</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
<td>&gt;64</td>
<td>8</td>
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<tr>
<td><strong>Rest of the World</strong></td>
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<td></td>
<td></td>
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<tr>
<td>All (1154)</td>
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<td>&gt;16</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
<td>64</td>
<td>&gt;8</td>
</tr>
<tr>
<td>ESBL (848)</td>
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<td>0.5</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
<td>64</td>
<td>&gt;8</td>
</tr>
<tr>
<td>AmpC (29)</td>
<td>4</td>
<td>&gt;16</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
<td>64</td>
<td>&gt;8</td>
</tr>
<tr>
<td>ESBL+AmpC (27)</td>
<td>1</td>
<td>16</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
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<tr>
<td>SerCp’ase (52)</td>
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<td>&gt;16</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
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<td>&gt;8</td>
</tr>
<tr>
<td>SerCp’ase+ESBL/AmpC (119)</td>
<td>2</td>
<td>&gt;16</td>
<td>&gt;32</td>
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<td>&gt;128</td>
<td>32</td>
<td>&gt;8</td>
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<tr>
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<td>4-&gt;16</td>
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<td>32-128</td>
<td>1-8</td>
<td>0.5-&gt;8</td>
</tr>
<tr>
<td>MBL+ESBL/AmpC² (22)</td>
<td>2</td>
<td>&gt;16</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
<td>64</td>
<td>&gt;8</td>
</tr>
<tr>
<td>OSBL or no bla gene detected (54)</td>
<td>2</td>
<td>&gt;16</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;128</td>
<td>64</td>
<td>&gt;8</td>
</tr>
</tbody>
</table>

TGC, tigecycline; IMI/MEM, imipenem or meropenem; FEP, cefepime; CAZ, ceftazidime; TZP, piperacillin-tazobactam; AMK, amikacin; LVX, levofloxacin.

ESBL, extended-spectrum \(\beta\)-lactamase; AmpC, plasmid-encoded class C \(\beta\)-lactamase; SerCp’ase, serine carbapenemase (KPC or OXA-48); MBL, metallo-\(\beta\)-lactamase; OSBL, original spectrum \(\beta\)-lactamases.

¹Ranges or individual MICs are provided where n <10.
²Includes isolates encoding ESBLs or AmpCs or both ESBLs and AmpCs.

**Conclusions**: Fewer carbapenem non-susceptible isolates were collected from Asia/Pacific than the rest of the world during 2005-2011. Regional differences in prevalence and presumed mechanism of carbapenem-resistance (production of carbapenemases, changes in permeability or efflux) were observed. Tigecycline continues to display significant *in vitro* activity against MDR *Enterobacteriaceae* collected globally.
Antimicrobial Susceptibility Profiles of Key Intra-Abdominal Bacterial Isolates from a Global Population

M. Hackel1, D. Biedenbach1, B. Johnson1, H. Leister-Tebbe2, I. Morrissey3

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2Pfizer Inc., Collegeville, PA, USA
3IHMA Europe Sàrl, Epalinges, Switzerland

Background: The empiric treatment of intra-abdominal infections (IAI) is complicated due to the changing epidemiology of pathogens and the emergence and evolution of antimicrobial resistance. Enterobacteriaceae, P. aeruginosa and A. baumannii are common causes of IAI, and increasingly exhibit resistance to many of the drugs indicated for their treatment. Intra-abdominal pathogens and resistance patterns vary geographically, therefore careful monitoring is warranted. In this analysis, IAI data from the Tigecycline Evaluation Surveillance Trial (TEST) were used to evaluate the in vitro activity of several key drugs against intra-abdominal isolates from a global population.

Methods: A total of 5,232 IAI clinically significant isolates were collected from Africa (242), Asia/Pacific (10), Europe (3,550), Latin America (182), Middle East (60) and North America (1,188) during 2011-2012. MICs were determined at each site by broth microdilution following CLSI guidelines and interpreted using current CLSI and FDA (tigecycline) breakpoints.

Results: The in vitro activity of select drugs are provided in the Table below.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Enterobacteriaceae (4,644)</th>
<th>P. aeruginosa (425)</th>
<th>A. baumannii (163)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% S</td>
<td>MIC50</td>
<td>MIC90</td>
</tr>
<tr>
<td>Amikacin</td>
<td>98</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Cefepime</td>
<td>89</td>
<td>≤0.5</td>
<td>16</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>77</td>
<td>≤1</td>
<td>&gt; 16</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>81</td>
<td>0.06</td>
<td>&gt; 8</td>
</tr>
<tr>
<td>Meropenem</td>
<td>97</td>
<td>≤0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>Pip-Tazo</td>
<td>85</td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>97</td>
<td>0.25</td>
<td>1</td>
</tr>
</tbody>
</table>

%S-percent susceptible; MIC50/90 in µg/ml; na-no breakpoint available

Conclusions: Amikacin, meropenem, and tigecycline were the most active agents against Enterobacteriaceae, with %S >90%. Amikacin was the most active agent against P. aeruginosa with all others having susceptibilities ≤80%. Based on MIC90 values, tigecycline exhibited the greatest activity against A. baumanii with an MIC90 value of 1 µg/ml, with the MIC90 values for all other agents exceeding the highest concentration tested.
Global Antimicrobial Resistance Patterns Among *Enterobacteriaceae* Isolated From Inpatients (TEST 2010-2013)

D. Biedenbach¹, D. Sahm¹, S. Bouchillon¹, H. Leister-Tebbe², I. Morrissey³

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²Pfizer Inc., Collegeville, PA, USA
³IHMA Europe Sàrl, Epalinges, Switzerland

**Background:** Over the last decade, there has been a shift in the resistance patterns observed among enteric pathogens. The emergence, spread and increasing prevalence of carbapenem resistant *Enterobacteriaceae* (CRE) has changed antimicrobial profiles for these species, particularly among hospitalized patients. Resistance patterns can vary geographically and this study documents susceptibility rates for inpatient pathogens in five regions.

**Methods:** Hospitals in Africa/Middle East (AFME), Latin America (LA), North America (NA), Europe (EU), and Asia-Pacific (APAC) contributed 10,741 enteric isolates from inpatients, including those in ICUs, in 2010-2013. Susceptibility (S) testing was performed by broth microdilution and MIC results were interpreted according to CLSI performance standards or FDA guidance (tigecycline).

**Results:** The activity of seven agents against entericspecies are shown in the table.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>All regions (N=10,741)</th>
<th>CRE (N=340)</th>
<th>APAC (N=492)</th>
<th>AFME (N=626)</th>
<th>EU (N=5,817)</th>
<th>NA (N=2,461)</th>
<th>LA (N=1,345)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone</td>
<td>67/&gt;64</td>
<td>4/&gt;64</td>
<td>57/&gt;64</td>
<td>58/&gt;64</td>
<td>66/&gt;64</td>
<td>80/&gt;32</td>
<td>54/&gt;64</td>
</tr>
<tr>
<td>Cefepime</td>
<td>85/32</td>
<td>19/&gt;32</td>
<td>80/&gt;32</td>
<td>75/&gt;32</td>
<td>84/&gt;32</td>
<td>94/&gt;4</td>
<td>77/&gt;32</td>
</tr>
<tr>
<td>Meropenem</td>
<td>97/0.25</td>
<td>95/0.25</td>
<td>94/0.25</td>
<td>97/0.12</td>
<td>98/0.25</td>
<td>96/0.5</td>
<td></td>
</tr>
<tr>
<td>Pip-tazo</td>
<td>81/128</td>
<td>11/&gt;128</td>
<td>82/64</td>
<td>79/128</td>
<td>79/128</td>
<td>89/&gt;32</td>
<td>72/&gt;128</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>75/&gt;8</td>
<td>69/&gt;8</td>
<td>68/&gt;8</td>
<td>76/&gt;8</td>
<td>82/&gt;8</td>
<td>62/&gt;8</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>96/8</td>
<td>59/&gt;64</td>
<td>91/16</td>
<td>96/8</td>
<td>97/8</td>
<td>99/4</td>
<td>93/16</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>97/2</td>
<td>93/2</td>
<td>96/2</td>
<td>94/2</td>
<td>96/2</td>
<td>97/1</td>
<td>97/2</td>
</tr>
</tbody>
</table>

**Conclusions:** Overall, CRE represented 3.2% with highest rates in AFME (7.2%), APAC (4.7%), and LA (4%) compared to EU (3%) and NA (2.2%). Co-resistance among CRE to other classes was significant with only amikacin and tigecycline having any significant *in vitro* activity. Overall antimicrobial S was highest in NA, levofloxacin and amikacin S was lowest in LA and APAC, respectively. Tigecycline maintained consistent activity across regions, but was lower among CRE.
Regional Variations in Antimicrobial Susceptibility Patterns
Among Acinetobacter baumannii Collected During TEST Surveillance 2012-2013

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2Pfizer Inc., Collegeville, PA, USA
3IHMA Europe Sàrl, Epalinges, Switzerland

Background: Acinetobacter baumannii (ACB) mostly cause infections among seriously ill, hospitalized patients and have been associated with high levels of resistance to multiple antimicrobial classes. Resistance patterns for this species vary geographically. This study, analysis of Tigecycline Evaluation Surveillance Trial (TEST) data documents susceptibility rates in five regions.

Methods: Hospitals in Africa/Middle East (AFME), Latin America (LA), North America (NA), Europe (EU), and Asia-Pacific (APAC) contributed 2,797 ACB isolates from multiple specimen sources in 2012-2013. Susceptibility testing was performed at each site by broth microdilution following CLSI guidelines and MIC results were interpreted using current CLSI and FDA (tigecycline) breakpoints.

Results: The activity of seven agents against ACB are shown in the table.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>All ACB (N=2,797)</th>
<th>MDR ACB (N=1,631)</th>
<th>APAC (N=471)</th>
<th>AFME (N=619)</th>
<th>EU (N=271)</th>
<th>NA (N=78)</th>
<th>LA (N=179)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Susceptible/MIC90 (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>39.2/&gt;16</td>
<td>4.9/&gt;16</td>
<td>6.1/&gt;16</td>
<td>9.1/&gt;16</td>
<td>42.3/&gt;16</td>
<td>49.8/&gt;16</td>
<td>8.5/&gt;16</td>
</tr>
<tr>
<td>Cefepime</td>
<td>37.5/&gt;32</td>
<td>1.8/&gt;32</td>
<td>6.1/&gt;32</td>
<td>7.1/&gt;32</td>
<td>40.9/&gt;32</td>
<td>47.5/&gt;32</td>
<td>7.3/&gt;32</td>
</tr>
<tr>
<td>Meropenem</td>
<td>43.2/&gt;16</td>
<td>4.3/&gt;16</td>
<td>25.0/&gt;16</td>
<td>10.4/&gt;16</td>
<td>46.2/&gt;16</td>
<td>54.0/&gt;16</td>
<td>9.9/&gt;16</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>38.1/&gt;8</td>
<td>2.6/&gt;8</td>
<td>15.2/&gt;8</td>
<td>13.9/&gt;8</td>
<td>39.8/&gt;8</td>
<td>48.7/&gt;8</td>
<td>6.1/&gt;8</td>
</tr>
<tr>
<td>Amikacin</td>
<td>58.5/&gt;64</td>
<td>30.4/&gt;64</td>
<td>18.2/&gt;64</td>
<td>32.3/&gt;64</td>
<td>58.0/&gt;64</td>
<td>75.5/&gt;64</td>
<td>25.6/&gt;64</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>NB/1</td>
<td>NB/2</td>
<td>NB/1</td>
<td>NB/1</td>
<td>NB/2</td>
<td>NB/2</td>
<td>NB/1</td>
</tr>
</tbody>
</table>

MDR, multidrug-resistant (>=3 classes); NB, no breakpoint criteria

Among the 2,797 ACB isolates collected 58.3% were MDR in all regions combined. MDR rates were higher in AFME, APAC, and LA (89-92%) compared to EU (56%) and NA (46%).

Conclusions: Overall, the susceptibility of ACB is low for all agents tested with no reliable drugs for treating MDR ACB, which comprised >=90% from three regions in this collection. Only tigecycline demonstrated in vitro activity, with MIC90 values ranging from 1 mg/L for all ACB to 2 mg/L for MDR strains, though not indicated for ACB therapy.
DETECTION RATE OF NONTUBERCULOUS MYCOBACTERIA: UiTM EXPERIENCE
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Background: In 1930s nontuberculous mycobacteria (NTM) were documented to cause disease in humans, but it was not until 1950s that pulmonary disease due to these organisms became more commonly recognized. The advance of Acquired immune deficiency syndrome (AIDS) contributed as the major milestone in understanding the clinical significance of these organisms in causing infection. Clinically important NTM includes M.marinum, M.simiae, M.srofulaceum, M.intracellulare, M.ulcerans, M.abscessus, M. szulgai, M. haemophilum, and M. genevense. There are four clinical syndromes associated with NTM and it comprises of pulmonary diseases, lymphadenitis, skin or soft tissue disease, and disseminated disease. Hence, this study conducted with the aim to investigate the rate and species of NTM isolated in Faculty of Medicine, UiTM.

Methodology: One thousand nine hundred and twenty clinical specimens were stained in Microbiology laboratory, CPDRL, and only one hundred and nine clinical specimens were stained and cultured from January 2011 to August 2014. Kinyoun stain was used to detect acid fast bacilli (AFB) in all specimens, and submitted to the University Malaya Medical Centre for further isolation and identification of NTM species.

Results: Of 109 clinical specimens cultured, there was 98.0% (n=107) of specimens consist of sputum and remaining 2.0 % (n=2) comprise of pus aspirate and tissue respectively. Out of 109 specimens, 24.9% (n=26) were culture positive and 76.9% (n=83) culture negative. Of 26 culture positive, 27.0% (n=7) of isolates was identified as NTM species and 73% (n=19) identified as Mycobacterium tuberculosis complex. Out of seven NTM, 57.0% (n=4) was identified as Mycobacterium abscessus and remaining 43.0% (n=3) identified as M. intracellulare, M. interjectum and M. fortuitum respectively. M. interjectum was isolated from the pus aspirate, while one out of four M. abscessus isolated from tissue. The other five of NTM species was isolated from sputum. Of seven NTM species isolated, 29.0% (n=2) , namely M. abscessus demonstrated strongly positive AFB (more than 50 AFB/length) in the Kinyoun stain, while others were negative for AFB.

Conclusion: There was evident in this study that NTM species was present, although the detection rate was low in contrast to Mycobacterium tuberculosis complex. Therefore, the need to identify NTM species remains vital as it causes significant clinical syndrome and the management vary depending on the species of NTM.
Clinical Profile of Adult Patients with Severe Sepsis: 
Real World Experience in Resource-Limited Borneo District Setting

TS Leong1, CW Ngua2, CS Chai1, YF Ho1, WK Wong1, SL Lai1, CH Liew1, Chin WV1

1Medical Department, Kapit District Hospital, Sarawak, Malaysia
2Emergency Department, Kapit District Hospital, Sarawak, Malaysia

Introduction:
Severe sepsis is defined as infection causing tissue hypo-perfusion. Mortality ranging from 10% to 50%. In 2013, more than 20% of adult patients admitted to Kapit Hospital, a district hospital in interior Borneo Sarawak, Malaysia were attributed to infection. About a quarter of them have severe sepsis. They will be admitted to a three bedded High Dependency Unit (HDU) in Kapit Hospital that is co-managed by a physician, ICU trained medical officer and nurses. It is equipped with basic facilities such as ABG, ventilators, and arterial monitoring. Its location of 130 km away from its tertiary center, accessibility only via a 3-hour express boat journey, poses unique challenges in managing patient with severe sepsis.

Objective:
1. To identify demographics, diagnosis and severity of patients treated as severe sepsis in Kapit Hospital.
2. To establish possible risk factors, treatment and outcome of patients treated as severe sepsis in Kapit Hospital.

Methodology:
A 4-month retrospective cohort study of adult patients treated as severe sepsis based on criteria of Surviving Sepsis Campaign (SSC) 2014 was undertaken for the first time in a Borneo district hospital setting. Adult patients admitted to HDU Kapit Hospital from April to August 2014 were captured and reviewed. Clinical profile, diagnosis, severity (based on APACHE II and SOFA), treatment and outcome were entered into pre-designed case report forms. Data was analysed and interpreted via IBM SPSS 20.0

Results/Discussion:
There were a total of 35 patients in our study with the male to female ratio of almost 1:1 (18:17). Mean age is 48.9 years (range 16 - 83). Eight of them have diabetes (22.9%). However, most of them do not have any risk factor (n=22, 62.9%). A high proportion of them have Gram negative sepsis (n=8, 22.9%), followed by malaria (n=7, 20%), gram positive sepsis (n=4,11.4%), leptospirosis (n=4, 11.4%), dengue (n=1, 2.9%) and tuberculosis (n=1, 2.9%). Ten patients (28.6%) had severe sepsis where the culture was negative. The mean APACHE II score was 16 (range 6 - 33). Mean SOFA score was 8.3 (3-13). Mean ICU length of stay was 4 (range 1 – 8 days). A high proportion of patients were treated with third/fourth generation cephalosporin (40%), followed by broad spectrum penicillin (22.9%), artesunate (20%) and carbapenem (11.4%). Mortality rate was 17.1% (n=6). Twenty nine patients required at least one inotropic support (82.9%). Nine of them (25.7%) required some form of ventilation support. A higher proportion of death was noted in patient with APACHE II score more than 16 (p= 0.007)

Conclusion:
Severe sepsis can be managed in district setting with basic invasive facilities as mortality rate is comparable with literature. Data should be gathered for a longer period to increase the power of the study. Correlation of adherence to SSC guideline 2014 with survival rate in a resource limited district setting should also be undertaken.
Pulmonary Melioidosis in Kapit Hospital, Sarawak: 
A Report of 5 Cases & Review

TS Leong, CS Chai, YF Ho

Medical Department, Kapit Hospital, Sarawak, Malaysia.

Introduction:
Melioidosis is a potentially fatal infection, caused by Burkholderia pseudomallei. It is a disease that is predominantly seen in Thailand, Malaysia, Northern Australia and India. Pulmonary melioidosis e.g. pneumonia is the most common clinical manifestation of melioidosis. Kapit district of Sarawak, Malaysia is an endemic hot spot for melioidosis.

Objective:
The aim of this review is to describe the spectrum, in terms of presentation, severity and outcome of culture proven pulmonary melioidosis treated in Kapit District Hospital.

Methodology:
Case notes of five cases of culture positive pulmonary melioidosis treated in Hospital were reviewed. The patient profile, potential risk factors, clinical features, investigation findings as well as eventual outcome were discussed in the form of case presentation.

Results/Discussion:
All patients present with symptoms such as fever, chills and rigors associated with productive cough for at least one week prior to seeking treatment. One particular patient presented with one week history of right knee joint effusion which was subsequently tapped and grew B.Pseudomallei. All the patients’ CXR have patchy consolidative changes with varying pattern of infiltrates. All the patients’ blood culture grew B.Pseudomallei; two patients’ sputum grew B.Pseudomallei. They presents with a spectrum of severity, ranging from respiratory distress needing intubation (n=3), septic shock requiring at least one inotrope (n=4), severe metabolic acidosis (n=2) and acute kidney injury needing dialysis (n=1). Three patients died despite escalation of treatment from ceftazidime to carbapenem and transfer to tertiary center with proper ICU care. Two patients completed standard treatment and were discharged well.

Conclusion:
Pulmonary melioidosis can present with varying spectrum of severity. Poor prognosis factor includes respiratory failure, renal failure and metabolic acidosis. High suspicion in endemic area such as Kapit district is crucial in ensuring early treatment and preventing mortality.
Comparison of Aerobic, Anaerobic and Pediatric Blood Culture Media and their Time-to-Positivity of simulated Candidamia.

M Hui*, FT Chan.
Department of Microbiology, the Prince of Wales Hospital, the Chinese University of Hong Kong, Hong Kong SAR

Background: Candidaemia is an important infectious disease with high mortality. Prompt diagnostic confirmation is desirable in guiding antimicrobial therapy. Candida glabrata had been reported to give shorter time-to-positivity (TTP) in anaerobic blood culture bottles. In this study, we aimed to investigate the TTP of different Candida species in aerobic, anaerobic and pediatric blood culture bottles. The effect of inoculum size was also assessed.

Method: One ATCC type strain and one clinical strain of the following 5 Candida species were tested: Candida albicans (ATCC 24433), Candida tropicalis (ATCC 750), Candida parapsilosis (ATCC 22019), Candida glabrata (ATCC 90030), and Candida krusei (ATCC 6258). Simulated blood samples with horse blood were spiked with concentrations of the various Candida species at $10^1$, $10^2$, $10^3$ and $10^4$ cells per mL. Five milliliters of simulated blood samples were spiked into individual aerobic and anaerobic blood culture bottles (BD BACTEC™ Plus Aerobic/F and Plus Anaerobic/F). Three milliliters of simulated blood samples were inoculated into pediatric blood culture bottles (BD BACTEC Peds Plus™/F). These bottles were incubated in blood culture system (BD BACTEC™ FX) for 14 days. TTP was recorded and all tests were done in duplicates. Continuous variables, categorical variables and correlation were analysed by SPSS. A $p$ value of < 0.05 was considered as significant.

Results: With the exception of C. glabrata, none of the Candida species showed growth in anaerobic blood culture bottles. The TTP for C. glabrata were significantly shorter in anaerobic culture bottles than in aerobic and pediatric bottles at all 4 concentrations ($p < 0.05$); the pediatric blood culture bottles took the longest TTP at all 4 concentrations ($p < 0.05$). For C. albicans, C. tropicalis, C. parapsilosis and C. krusei, no significant difference were observed in TTP between aerobic and anaerobic cultures ($p < 0.05$). Increasing concentrations were correlated significantly with shortening of TTP ($Pearson r = -0.834$).

Conclusion: C. glabrata is capable of growing in anaerobic blood culture bottles and at a faster rate than in aerobic or pediatric blood culture bottles. This can act as an early clue to the presence of potentially azole resistant C. glabrata in positively flagged blood culture bottles. The shorter duration of TTP of all 5 Candida species would suggest a larger inoculum in the original blood samples. This finding can be further explored for its prognostic significance.

(423 words)
Antibacterial Effects of Highly Alkaline Electrolysed Water.

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Department of Microbiology, Department of Surgery, the Prince of Wales Hospital, the Chinese University of Hong Kong, Hong Kong SAR

Background: Multidrug resistant bacteria have emerged as important human pathogens. They can colonized the patient’s immediate environment and act as potential source of nosocomial spread. Environmental disinfection with chemical disinfectants is not always effective. There are also safety concerns with the use of chemical disinfectants such as chlorine-containing agent. In this study we aimed to investigate the antibacterial effects of electrolyzed water (EW) with highly alkaline pH.

Method: Type strains of *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella typhimurium* (ATCC 13311) and *E. coli* O157:H7 (ATCC 35150) were used. EW was obtained by electrolysis of H₂O and K₂CO₃ at pH 12.0 – 12.5 (Ewash, Eplan Japan). Bacterial suspension tests were performed by suspending the bacteria into the EW to a final concentration of 10⁸/mL. It was then allowed to stand at 25°C in water bath for 5 min. These treated bacterial suspensions were then enumerated by viable plate count. All tests were done in duplicates and replicated. Descriptive statistics was used where appropriate.

Results: The EW was capable of producing 7 log₁₀ reduction in bacterial suspension tests for type strains of *Pseudomonas aeruginosa, Salmonella typhimurium* and *E. coli* O157:H7. The reduction for *Staphylococcus aureus* was less than 1 log₁₀.

Conclusion: The EW is effective as a bactericidal agent for Gram negative bacteria. This finding can be explored for its potential usage as disinfectant. The lack of effect on *Staphylococcus aureus* suggests that it may be less effective in Gram positive bacteria. The effect of combining EW with other disinfectants can be explored to improve its efficacy.

(292 words)
Genus and species distribution of *Enterobacteriaceae* causing non-enteric community acquired infections

Syahrul Azlin Shaari, Norazian Ibrahim*, Fadzilah Mohd Nor, Noraziah Sahlan

*Medical Microbiology unit, Centre for Pathology & Diagnostic Research Laboratories (CPDRL), Faculty of Medicine, Universiti Teknologi MARA*

*Enterobacteriaceae* is a group of gram-negative bacilli consisting of over 30 genera and 120 species. However, clinically significant strains fall into only 10 genera and 25 species. Out of this 25 species, three of which constitutes up to 95% of all isolates identified in clinical setting. It has been widely reported that the most common pathogen is *Escherichia coli*, followed by *Klebsiella pneumoniae* and thirdly *Proteus mirabilis*.

A retrospective study was done on gram-negative bacteria recovered from non-enteric-related clinical specimen, of patients admitted to Clinical Training Centre, Faculty of Medicine UiTM in the years of 2012 and 2013. Data for specimens which were sampled after forty-eight hours of patients’ admission were excluded. All gram-negative isolates were standardly identified using VITEK 2 GN colorimetric identification card in Centre for Pathology and Diagnostic Research Laboratories. Only isolates identified with >95% confidence were selected. One hundred and two *Enterobacteriaceae* fulfilled the inclusion criteria and included in the analysis.

*Enterobacteriaceae* which contributes to infections in this group of patients were made up of six genuses, five of which were coliforms and the other one a non-coliform. They were the *Klebsiella*, *Escherichia*, *Enterobacter*, *Serratia*, *Proteus* and *Citrobacter*. The most commonly isolated pathogen was *Klebsiella pneumoniae* (37.3%), and out of this percentage *Klebsiella pneumoniae* subsp. *pneumoniae* was the dominant subspecies (n = 37) in comparison to *Klebsiella pneumoniae* subsp. *ozoaenae* (n = 1). The next most common *Enterobacteriaceae* was *Escherichia coli* (35.3%); followed by the *Enterobacter*, *Enterobacter cloacae* and *Enterobacter aerogenes* (10.7%), the *Serratia*, *Serratia marcescens* and *Serratio fonticola* (6.9%), *Proteus mirabilis* (5.9%) and *Citrobacter freundii* (3.9%). The types of infections caused by these various organisms ranges from a simple abscess to bacteraemia, and everything in between. *Klebsiella pneumoniae* were most frequently recovered from respiratory specimens, whereas *Escherichia coli* dominated recovery from urinary specimens. Representative isolates from all six genera were recovered from blood culture specimens.

This study confirms that *Klebsiella pneumoniae* and *Escherichia coli* still remain as the two most important *Enterobacteriaceae* causing infections. However, it suggests that *Klebsiella pneumoniae* is an equally, if not a more significant pathogen compared to *Escherichia coli*. Another significant contradictive finding is that *Enterobacter* is a more common pathogen than previously reported. Nevertheless, this study concur that *Enterobacter cloacae* is the more frequently implicated species than *Enterobacter aerogenes*. For spectrum of infections, while the *Klebsiella* and *Escherichia* are mostly associated with urinary tract and respiratory tract infections respectively, and all six genera are capable of causing serious infections such as bacteraemia.
PATTERN OF DRUG RESISTANCE MUTATIONS AMONG HIV-1 PATIENTS WITH VIROLOGICAL FAILURE IN MALAYSIA.

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Background:
Currently, the data on HIV-1 drug resistance mutation (DRM) is very limited in Malaysia. This data is seriously needed to guide physicians in planning the most effective antiretroviral (ARV) therapy (ART) regime for HIV-1 infected patients who have developed resistance towards the drug treatment. Hence, the aim of this study was to determine the most common HIV-1 genomic mutation(s) and subtype(s) among HIV-1 patients with virological failure in Malaysia.

Methods:
A total of 75 HIV-1 patients experiencing virological failure were selected from four hospitals accommodated with viral load facility viz. Hospital Sultanah Aminah, Hospital Raja Perempuan Zainab II, Hospital Sungai Buloh and Hospital Pulau Pinang. Plasma samples were collected from these patients and all samples with viral load of >2000 copies/ml were subjected to the in-house HIV-1 genotyping assay. The entire HIV-protease and up to codon 335 of the HIV-reverse transcriptase regions were sequenced to cover all known DRMs. The results were then submitted to the Stanford HIV-1 Drug Resistance Database to yield associated DRMs and for subtype analysis.

Results:
All samples were amplified and sequenced successfully. DRMs were detected in 68 patients (90.7%). Major resistance mutations towards Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs) occurred in 56 (82.4%), 61 (89.7%) and 2 (2.9%) patients, respectively. Both patients in the latter also exhibited NRTI mutations that may confer soaring resistance towards multiple ARV agents. The existence of PI Minor DRMs was also observed in 20 (29.4%) patients. Of these, 25% were L10I and 25% were L10V mutations. The most common DRMs found to NRTI, out of 48 different DRMs detected, were found to be M184V with 43 (89.6%) patients and K70R with 10 (20.8%) patients. From the 42 patients with different DRMs detected to NNRTI, K103N, Y181C and P225H with 30 (71.4%), 17 (40.5%) and 11 (26.2%) patients, respectively, were the most common. Subtype analysis showed that CRF01_AE at 84% (63/75) was the most predominant HIV-1 subtype among the group of patients studied.

Conclusion:
The vast detection of DRMs in this study emphasized the importance of genotypic resistance test in the management of HIV patients as DRMs can alter patient’s susceptibility towards ARV drugs. Further study on larger number of samples is essential for the development of a database on HIV-1 DRMs among patients that experience virological failure in Malaysia.
Carbapenem Resistance in ESBL-producing *E.coli*

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Background: Extended-spectrum β-lactamases (ESBL)-producing *E.coli* is a clinical threat that may cause nosocomial as well as community-acquired urinary tract infection. Carbapenems are a unique class of β-lactam antibiotics with the widest spectrum of antibacterial activity among the currently available antibiotics and are considered the last resort for treatment of ESBL producing *E.coli*. Carbapenemases are enzymes that can recognize carbapenems and almost all hydrolysable β-lactams.

Objectives: Molecular confirmation of ESBL production and phenotypic detection of carbapenemases among phenotypically proved ESBL-producing *E.coli*.

Methods: Forty ESBL-producing *E.coli* isolated mainly from urine samples were tested for resistance to carbapenems with initial screening using meropenem disc diffusion test and imipenem E-test. The modified Hodge test and the E-test MBL IP/IPI were done. Then the isolates were subjected to multiplex PCR for detection of genes encoding ESBLs using primers for *bla*-TEM, *bla*-CTX-M1, *bla*-CTX-M2, *bla*-SHV and *bla*-PER genes.

Results: thirty five (87.5%) out of 40 isolates harboured one or more β-lactamases genes. *bla*-TEM gene was predominantly detected in 22 isolates (55%) followed by *bla*-CTX-M1 (45%) and *bla*-PER (32.5%). *bla*-SHV and *bla*-CTX-M2 were the least detected genes (1% and 0.75% respectively). None of the ESBL-producing *E.coli* was positive for carbapenemases.

Conclusion: ESBL are expressed by different β-lactamases genes. Carbapenemases production was not found in any of the tested ESBL-producing *E.coli*.

Key words: *E.coli*, ESBL, *bla*-TEM, *bla*-CTX-M1, *bla*-CTX-M2, *bla*-SHV, *bla*-PER, carbapenemases.
Prevalence of Quinolone High-Resistant and ESBL Producing *Proteus mirabilis* in western part of Japan

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**Background:** *Proteus mirabilis* strain does not have any beta-lactamase in its chromosomal DNA. So cephems-resistant *P. mirabilis* isolates were rare. However, ESBL producing isolates appeared and have been increasing since 2002 in Western part of Japan area. Levofloxacin resistant *P. mirabilis* isolates have also been increasing. We performed a survey of resistance to various antibiotics in *P. mirabilis* isolated from clinical specimen in Western part of Japan.

**Methods:** A total of 955 non-duplicate *P. mirabilis* isolated from clinical specimen in the period from 2008 to 2013 in 121 participating centers were collected. The MICs of various antimicrobials against the isolates were determined by the two-fold serial agar dilution method as described by the CLSI. The resistant isolates to antimicrobials were basically interpreted as showing more than following breakpoint of susceptible category. ESBLs were confirmed phenotype using clavulanic acid, and type of ESBLs were determined by PCR method using specific primers.

**Results:** Urine sample, respiratory sample, and pus accounted for 58.0, 29.9, and 7.6%, respectively. The isolates from inpatients accounted for 70.4%. The ratio of ESBL producing isolates and levofloxacin-resistant isolates in 2008, 2009, 2010, 2011, 2012, and 2013 were 29.9, 27.0, 24.9, 34.2, and 29.8%, and 49.3, 45.9, 42.8, 45.7, 49.6, and 38.5%, respectively. Levofloxacin-resistant isolates accounted for 87.9% in ESBL producing isolates. CTX-M-2 type accounted for 98.2% (279/284) in ESBL producing isolates. The ratio of CMY-2 that is class C beta-lactamase, producers in 2008, 2009, 2010, 2011, 2012, and 2013 were 0.5, 0.5, 1.2, 2.5, 2.6, and 0.6%, respectively. Two isolates had CTX-M-2 and CMY-2 beta-lactamase.

The resistant ratios (more than value in parenthesis) of ampicillin (8), piperacillin (16), cefazoline (2), cefpodoxime (2), ceftriaxone (1), cefazidime (4), cefpirome (2), cefmetazole (16), imipenem (1), gentamicin (4), amikacin (16), and isepamicin (16), levofloxacin (2), sitafloxacin (0.5), fosfomycin (64), and trimethoprim/sulfamethoxazole (2/38) against 254 levofloxacin-resistant and ESBL producing isolates were 100, 98.4, 100, 100, 12.2, 0.8, 100, 0.4, 5.9, 3.1, 3.7, 3.1, 97.2, and 5.5%, respectively. There is no isolates that have higher MIC to piperacillin/tazobactam (16), flomoxef (8), aztreonam (4), and meropenem (1) than their breakpoint MICs.

**Conclusions:** It is serious problem that ESBL producers accounted for about 30%, and levofloxacin-resistant isolates accounted for about 45%. Moreover, levofloxacin-resistant ESBL producers accounted for 26.6%. Clinical problem concerning infectious diseases caused by this multi-drug resistant *P. mirabilis* have not occurred yet. But near future, clinical chemotherapy, especially oral therapy, will be complicated by the multi-drug resistant *P. mirabilis*. 
Risk factors for mortality in invasive pneumococcal disease in Singapore

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Introduction: Invasive Streptococcus pneumoniae disease remains as an important cause of morbidity and mortality in Singapore even after the introduction of pneumococcal vaccines. A retrospective study reviewing all patients with invasive pneumococcal disease admitted to National University Hospital, Singapore, between December 2008 and March 2013 was conducted.

Methods: Clinical and laboratory data from all patients with Streptococcus pneumoniae isolated from sterile sites from December 2008 to March 2013 were reviewed. Serotyping and susceptibility testing were done by standard methods.

Results: A total of 119 patients with positive pneumococcal isolates were identified with a mean age of 50 ± 25.26 years. Fifteen (12.6%) were children <15 years of age, none of whom died. Of the 119 isolates, 110 (92.4%) were bloodstream isolates, 8 (6.7%) from cerebrospinal fluid and 1 (0.8%) from pleural fluid and other sterile sites. Radiologically confirmed pneumonia was the primary site of infection in 88 (74.0%). Other sites included: meningitis (8, 6.7%) meningitis, peritonitis (1, 0.8%) and pyomyositis (1, 0.8%). Twelve (10.1%) patients had pneumococcal bacteremia without an obvious primary source. Ninety-three (78.2%) isolates were penicillin-sensitive, while twenty-one (17.6%) were intermediate and five (4.2%) were resistant to penicillin. Ninety (75.6%) of the isolates were successfully serotyped. The commonest serotypes were 3, 6A, 6B, 8, 14, 19A, 19F, 23A, and 23F, making up 71.1%. The 23-valent pneumococcal polysaccharide vaccine (PPSV23) would have covered 91.1% of the serotypes, while the 13-valent pneumococcal conjugate vaccine (PCV-13) would have covered 74.4% of the serotypes. Overall Mortality was 13.5%. Risk factors for mortality identified included increased age (mean age of fatal cases 63.6 ± 20.5 years vs survivors 48.0 ± 25.5 years, p=0.02), serotype 3 (3/8 (37.5%) fatalities vs 8/82 (9.8%) for the other serotypes, p=0.02), leukopenia (white cell count < 3.5 X10^9/L on admission, 5/13 (38.5%) fatal cases vs 11/106 (10.4%) in survivors, p<0.01). Leukocytosis (white cell count > 15X10^9/L was protective (Odds ratio 0.22, 95% CI 0.04-0.92, p=0.03). Factors such as penicillin non-susceptibility, diabetes mellitus, anemia and ischemic heart disease did not affect mortality.

Conclusion: Penicillin susceptible invasive pneumococcal disease continues to cause significant mortality and morbidity in Singapore. Greater efforts need to be taken to increase vaccine uptake to prevent this potentially preventable infection.
Carbapenems may be the optimal empiric antibiotics to treat suspected *Escherichia coli* or *Klebsiella pneumoniae* infections

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Background
*Escherichia coli* and *Klebsiella pneumoniae* are two most common gram-negative bacilli resulting in various infections in medical practice. The incidence of extended-spectrum beta-lactamase (ESBL)-producing strains of both is increasing worldwide, which may change empiric antibiotic treatment. This study was conducted to explore the increasing extent of the incidence of ESBL-producing strains in *E. coli* and *K. pneumoniae*, and whether empiric antibiotic agents should be changed to antibiotics with activity against ESBL-producing strains, especially carbapenems.

Methods
This was a retrospective study at a regional hospital in southern Taiwan. From 2009 to 2013, all *E. coli* and *K. pneumoniae* isolates from clinical microbiology laboratory were enrolled in this study. ESBL-producing strains were confirmed by double disk diffusion test.

Results
A total of 15954 *E. coli* and 5374 *K. pneumoniae* isolates were enrolled. The ESBL-producing strains accounted for 10.7% (325 of 3048) and 16.8% (186 of 1109), 12.1% (362 of 2989) and 16.1% (155 of 963), 13.3% (447 of 3359) and 19.2% (214 of 1112), 17.4% (633 of 3635) and 20.8% (219 of 1051), as well as 20.5% (753 of 2923) and 20.2% (230 of 1139) of *E. coli* and *K. pneumoniae* isolates in 2009, 2010, 2011, 2012, and 2013, respectively. The incidence of ESBL-producing *E. coli* and *K. pneumoniae* was statistically significant difference between 2009 and 2013 (P < 0.05).

Conclusion
As a result of this study, the incidence of ESBL-producing strains of *E. coli* and *K. pneumoniae* was statistically significant increase from 2009 to 2013 (10.7% versus 20.5% in *E. coli* and 16.8% versus 20.2% in *K. pneumoniae*). In theory, empiric antibiotics should have activity against at least 80% of the likely pathogens; hence, carbapenems may be the most optimal therapy option for empiric therapy of infections suspected *E. coli* or *K. pneumoniae*, especially severe infections, in this hospital. In addition, implementing effective infection control measures are necessary to reduce the incidence of ESBL-producing strains of *E. coli* and *K. pneumoniae*. 
Clinical features and risk factors of culture positive pneumococcal pneumonia in Nagasaki, Japan

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Background: *Streptococcus pneumoniae* is an important bacterium in humans, colonizing the respiratory tract and a major etiology of variable infection. The aim of this study is to evaluate the clinical features, risk factors and serotype prevalence of pneumococcal infection in adults. **Methods:** The clinical manifestation, serotype distribution and of pneumococcal infections in adults (over 20 years), defined as clinical findings and culture result of clinical samples, were retrospectively analyzed in Nagasaki University Hospital from 2009 to 2013. **Results:** A total of 80 pneumococcal infections were collected. Mean age: 63.7±15.6 years, gender (male; 64.1%, female; 35.9%), laboratory findings (WBC 9882±6027 /mm³, CRP 7.13±9.12 mg/dl). Pneumonia (71.2%) was the main clinical syndrome (community-acquired pneumonia; 31.3%, hospital-acquired pneumonia; 23.8%, nursing and healthcare-associated pneumonia; 16.3%), while cancer, chronic kidney diseases, chronic heart diseases and diabetes mellitus were the main underlying diseases. 3 of 80 (4.3%) and 61 of 80 (76.3%) were resistant to penicillin and macrolide, respectively. Pneumococcal isolates were serotyped by latex agglutination and Quellung reaction. 33 of 80 cases were able to perform serotyping. PCV13 serotypes accounted for 72.7% of isolates, the most prevalent being serotypes 6 (27.3%), 19 (12.1%), 3 (9.1%), 4 (6.1%), 14 (6.1%) and 23 (6.1%). The malignant disease, especially hematological cancer was the independent risk factors for IPD (OR 35.429, 95% CI 3.459-362.857). **Conclusion:** These results indicated that hematological malignancy is the main risk factor for IPD in adults, suggesting the prophylaxis by PCV13 might be more recommended.
Assessing risk associated with methicillin-resistant Staphylococcus aureus acquisition in a multidisciplinary ward in Singapore

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Background
Multiple interventions have been undertaken to prevent methicillin-resistant Staphylococcus aureus (MRSA) transmission in hospitals. Little is known about the effect of universal octenidine bathing on MRSA acquisition.

Methods
We conducted an analytic cross-sectional study on patients admitted to the infectious disease and dermatological ward at the Communicable Disease Centre in Singapore. Daily bathing with octenidine was implemented for all patients for second half of the study period.

Results
During the period Jan-Dec 2013, the average MRSA prevalence of 940 screened patients at admission was 7.7% and acquisition was 2.4%. Sixteen case-patients screened negative for MRSA on admission and positive at discharge, and 554 control-patients screened negative for MRSA both on admission and at discharge were compared. Cases tend to have more hospitalizations in the preceding year before admission (odds ratio [OR], 2.9; 95% confidence interval [CI], 1.00-8.59), more co-morbidities with Charlson score>3 (OR, 2.09; 95%CI, 0.71-6.15), and from nursing homes (OR, 9.75; 95%CI, 1.90-50.15) respectively. After adjusting for age, gender, ethnicity, Charlson co-morbidity index, hospitalization in the preceding year, MRSA colonization pressure in the ward, hand hygiene compliance rate, surgery and antibiotic exposure during the hospital stay, total length of hospital stay, and daily universal octenidine bathing, nursing home residency status is the only factor independently associated with MRSA acquisition in the hospital (adjusted OR, 7.48; 95%CI, 1.02-55.01; P=0.048).

Conclusions
Nursing home residents are at increased risk for MRSA acquisition in the hospital, and may require enhanced infection prevention measures. A longer study period is required to better assess the impact of daily octenidine bathing on MRSA acquisition.
Background: Salmonellae are one of the most common bacterial pathogens causing food-borne diseases, and Salmonella entericaserovarEnteritidis is the most common, according for 65% of all human salmonellosis cases worldwide. This study was performed to investigate the multilocus sequence typing (MLST) of the CTX-M-type extended-spectrum \(\beta\)-lactamase (ESBL)-producing \textit{S}.\textit{enterica}\textit{serovarEnteritidis} clinical isolates from Korea.

Materials and methods: A total of 93 consecutive nonduplicate clinical isolates of \textit{S}.\textit{enterica}\textit{serovarEnteritidis} were collected from various clinical specimens between January and December 2008 at 12 tertiary-care hospitals in Korea. Antimicrobial susceptibility tests were performed using the disk diffusion test method according to Clinical and Laboratory Standards Institute guideline. \textit{Bla} gene amplification and nucleotide analysis were performed by Polymerase chain reaction and sequencing. MLST was performed using the 7 conserved housekeeping genes (\textit{aroC}, \textit{dnaN}, \textit{hemD}, \textit{hisD}, \textit{purE}, \textit{sucA}, and \textit{thrA}) following the protocols available at a certain website (http://mlst.warwick.ac.uk/mlst/dbs/Senterica).

Results: A total of 93 \textit{S}.\textit{enterica}\textit{serovarEnteritidis} strains were isolated from feces (81.7%; \(n=76\)), blood (15.0%; \(n=14\)), pus (1.1%; \(n=1\)), abscess (1.1%; \(n=1\)), and ascite (1.1%; \(n=1\)). Twentytwo (23.6%) in 90 \textit{S}.\textit{enterica}\textit{serovarEnteritidis} isolates exhibited resistance to both ceftazidime and cefotaxime. CTX-M-15 (54.5%; \(n=12\))
was most common ESBL, followed by CTX-M-14 (9.1%; n=2), and CTX-M-3 (4.5%; n=1). MLST analysis revealed two sequence types (STs) under one clonal complex (CC), including overrepresentation of CC4 and one new ST (ST1879).

Discussion: The present data showed the there was a clonal spread by *S. enterica* serovar Enteritidis CC4 producing CTX-M-type ESBLs in Korea in 2008.

Keywords: *Salmonella enterica* serovar Enteritidis, multilocus sequence typing, CTX-M
ASSESSMENT OF IN VITRO ANTIBACTERIAL ACTIVITIES OF BLACK CUMIN SEED (NIGELLA SATIVA) OIL BY MICRODILUTION METHOD

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Background: The in vitro antibacterial activities of N. sativa oil were evaluated against following strains; Staphylococcus aureus American Type Culture Collection (ATCC) 29213, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Pseudomonasaeruginosa ATCC 27853,45 clinical isolates of methicillin resistant S. aureus (MRSA) and 77 clinical isolates of methicillin resistant coagulase negative staphylococci (MRCoNS). The minimal inhibitory concentrations (MICs) of N. sativa oil were determined by broth microdilution method.

Methods: A real-time cell analyzer was used to evaluate the effects of different dilutions (0,25 µg/mL, 0,5 µg/mL and 1 µg/mL) of N. sativa oil on the proliferation of gingival fibroblasts. After seeding 200 µl of the cell suspensions in DMEM containing 10% fetal bovine serum (FBS) into wells (10,000 cells/well) of the E-plate 16, gingival fibroblast cells were monitored every 15 min for a period of 114 hours. Cells were allowed to adhere to the E-plate for 19 hours after seeding; the cells were exposed to 100 µL of medium containing dilutions of the N. sativa oil.

Results: The MIC values of N. sativa oil against S. aureus ATCC 29213, E. faecalis ATCC 29212, Escherichia coli ATCC 25922 and Pseudomonasaeruginosa ATCC 27853 standard strains were 0,5 µg/mL, 2 µg/mL, 64 µg/mL and 64 µg/mL respectively. The MIC values of N. sativa oil against clinical isolates of Staphylococci were between <0,25 µg/mL and 1,0 µg/mL. Compared to the control group, no cytotoxic effect on the proliferation of gingival fibroblasts was observed for the N. sativa oil applications.

Conclusion: As a result, the oil of N. sativa was very active against MRSA and MRCoNS in the present work and had no in vitro cytotoxicity at the relevant dilutions. There is need for further clinical trials for medical management of infections caused by methicillin resistant Staphylococci.
Ceftaroline fosamil is superior to ceftriaxone in the treatment of CAP: comparison of results from a trial with Asian patients to previous trials


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**Background:** Ceftaroline fosamil (CPT-F) 600mg q12h has been studied versus ceftriaxone (CRO) in community acquired pneumonia (CAP) in 3 randomised Phase III trials. A comparison of the results from the Asian study (CRO 2g q24h used as the comparator) and the other trials (CRO 1g q24h used as comparator) is presented.

**Methods:** Three Phase III trials of CPT-F vs CRO have been conducted: FOCUS1 (NCT00621504), FOCUS2 (NCT00509106), and Asia CAP (NCT01371838). The primary endpoint was the clinical response at the test-of-cure (TOC) in the clinically evaluable (CE) population (all trials) and in the PORT 3–4 modified intent-to-treat (MITT) population (FOCUS trials - referred to as MITTE in these trials). Secondary endpoints included clinical cure rates in the microbiological modified intent-to-treat (mMITT) and microbiologically evaluable (ME) populations as well as at end of treatment (EOT). A fixed effect meta-analysis using the inverse variance method was performed of the odds ratio (OR) of CPT-F vs CRO in clinical cure at TOC observed in each trial.

**Results:** Clinical cure rates at TOC in the CE population in the Asia CAP study were: CPT-F 84.1%, CRO 74.2%, difference (95% CI) 9.9% (2.8%, 17.1%); and in the MITT population CPT-F 80.1%, CRO 67.0%, difference (95% CI) 13.0% (6.8%, 19.2%), demonstrating the superiority of CPT-F to CRO. Equivalent data from the pooled FOCUS trials were (CE population): CPT-F 84.3%, CRO 77.7%, difference (95% CI) 6.7% (1.6%, 11.8%); (MITT population): CPT-F 82.6%, CRO 76.6%, difference (95% CI) 6.0% (1.4%, 10.7%), illustrating that the Asia CAP data were consistent with the FOCUS results, and that increasing the dose of CRO to 2g q24h did not have an impact on the clinical cure rate. Similar results were seen in the mMITT and ME populations and at EOT. A meta-analysis showed the results were consistent between trials with no evidence of heterogeneity and CPT-F was superior to CRO (e.g. CE population: OR 1.65, 95% CI 1.26, 2.16, p<0.001).

**Conclusion:** CPT-F is superior to CRO in the treatment of patients with PORT 3–4 CAP. Consistent results were seen in a trial in Asian CAP patients and the previously reported FOCUS trials.

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**Suggested keywords (3 permitted):** ceftaroline fosamil, efficacy, pneumonia

**Suggested subject category:** Bacterial infections: clinical epidemiology, antimicrobial resistance, therapy, outcome, and pathogenesis

**Presentation type**

- Oral or poster
Ceftaroline fosamil is superior to ceftriaxone in the treatment of CAP: comparison of results from a trial with Asian patients to previous trials

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Methods: Three Phase III trials of CPT-F vs CRO have been conducted: FOCUS1 (NCT00621504), FOCUS2 (NCT00509106), and Asia CAP (NCT01371838). The primary endpoint was the clinical response at the test-of-cure (TOC) in the clinically evaluable (CE) population (all trials) and in the PORT 3–4 modified intent-to-treat (MITT) population (FOCUS trials - referred to as MITTE in these trials). Secondary endpoints included clinical cure rates in the microbiological modified intent-to-treat (mMITT) and microbiologically evaluable (ME) populations as well as at end of treatment (EOT). A fixed effect meta-analysis using the inverse variance method was performed of the odds ratio (OR) of CPT-F vs CRO in clinical cure at TOC observed in each trial.

Results: Clinical cure rates at TOC in the CE population in the Asia CAP study were: CPT-F 84.1%, CRO 74.2%, difference (95% CI) 9.9% (2.8%, 17.1%); and in the MITT population CPT-F 80.1%, CRO 67.0%, difference (95% CI) 13.0% (6.8%, 19.2%), demonstrating the superiority of CPT-F to CRO. Equivalent data from the pooled FOCUS trials were (CE population): CPT-F 84.3%, CRO 77.7%, difference (95% CI) 6.7% (1.6%, 11.8%); (MITTE population): CPT-F 82.6%, CRO 76.6%, difference (95% CI) 6.0% (1.4%, 10.7%), illustrating that the Asia CAP data were consistent with the FOCUS results, and that increasing the dose of CRO to 2g q24h did not have an impact on the clinical cure rate. Similar results were seen in the mMITT and ME populations and at EOT. A meta-analysis showed the results were consistent between trials with no evidence of heterogeneity and CPT-F was superior to CRO (e.g. CE population: OR 1.65, 95% CI 1.26, 2.16, p<0.001).

Conclusion: CPT-F is superior to CRO in the treatment of patients with PORT 3–4 CAP. Consistent results were seen in a trial in Asian CAP patients and the previously reported FOCUS trials.

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Suggested keywords (3 permitted): ceftaroline fosamil, efficacy, pneumonia

Suggested subject category: Bacterial infections: clinical epidemiology, antimicrobial resistance, therapy, outcome, and pathogenesis

Presentation type

- Oral or poster
Association of virulence factors with phylogenetic groups of *Escherichia coli* isolated from UTI patients from Badin, Pakistan

N Kumar, F Nahid, R Zahra*

Department of Microbiology, Quaid-i-Azam University, Islamabad 45320, Pakistan

**Objective:** *Escherichia coli*, the most frequent cause of UTIs carry cluster of highly virulent genes and there exists an extensive genetic substructure within the species *Escherichia coli*. Using quadruplex PCR method described by Clermont et al., 2013 *E. coli* can be assigned to eight phylogroups, A, B1, B2, C, D, E, F and *Escherichia* cryptic clade I which give an insight into origin of these strains. In the present study, we investigated the distribution of virulence determinant genes of uropathogenic *E. coli* isolates in relation to eight phylogenetic groups.

**Methodology:** A total of 99 uropathogenic *E. coli* isolates were collected from Badin region of Sindh, Pakistan. Isolates were assigned phyllogroups using quadruplex PCR and PCR was performed for the detection of virulence genes *papC, sfa, fyuA, papGI, papGII, papGIII, hly, cnf and aer*. Cnf positive isolates were further screened for *hragene*. 

**Results:** Thirty four isolates were assigned to group B2, while 23, 2, 1, 7 and 22 isolates belonged to groups F, B1, A/C, clade I/II and unknown, respectively. Prevalence of *papC* (79%) was highest followed by *aer* (60%), *papGII* (13%), *papGIII* (11%), *cnf* (8%), *hly* (4%) and *sfa* (1%). Three isolates were positive for *hragene*.

**Conclusion:** This data provides the prevalence of phylogenetic groups and virulence associated genes in uropathogenic *E. coli* strains causing infection in Badin, Pakistan.
Prevalence of inducible clindamycin resistance in *Staphylococci* isolates at a tertiary care hospital from Islamabad, Pakistan

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**Objective:** The emergence of antibiotic resistant *Staphylococci* is an increasing problem and among available options, clindamycin is an appropriate choice for treating different *Staphylococcal* infections. The routine antibiotic susceptibility test is not suitable to detect inducible clindamycin resistance, hence misleading the clinicians. The objective of this study was to investigate the prevalence of clindamycin inducible resistance among clinical isolates of *Staphylococci.*

**Methodology:** The clinical isolates included in this study were collected from Pakistan Institute of Medical Sciences (PIMS), Islamabad. Bacterial identification was done by colony morphology and biochemical tests. Kirby-Bauer disc-diffusion method was used to assess the susceptibility pattern of *Staphylococci* against different antibiotics. The results were interpreted following Clinical and Laboratory Standards Institute (CLSI)2013 guidelines. Double Disk Diffusion test (D-test) was carried out to evaluate the clindamycin inducible resistance.

**Results:** A total of 176 clinical isolates of *Staphylococci* were collected from different sample source which were blood, pus, urine, sputum, tracheal secretions and tissue fluids. Prevalence of clindamycin inducible resistance among *Staphylococci* was found to be 7.23%, whereas in *Staphylococcus aureus* it was 5.12%, and for coagulase negative *Staphylococci* it was 9.45%. Resistance rate to other antibiotics were as following: cefoxitin 82.38%, linezolid 62.5%, vancomycin 13.06%, tigecycline 35.79%, tetracycline 58.52%, ciprofloxacin 54.54%, rifampin 35.22%, fosfomycin 92.61%, fusidic acid 84.09%, clindamycin 44.31%, erythromycin 53.97%, gentamicin 54.54%, chloramphenicol 18.74% and for sulfamethoxazole it was 53.40%. **Conclusion:** Inducible resistance is contributing towards clindamycin failure because clinicians prescribe clindamycin for different *Staphylococcal* infections without the knowledge of clindamycin inducible resistance. So it is suggested that D-test should be included in routine diagnostic tests to avoid clindamycin failure.
Salmonella Typhi Leg Abscess in a Patient with Myelodysplastic Syndrome
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Philippine General Hospital, Philippines

We describe a 59-year old male diagnosed with myelodysplastic syndrome (MDS) one year prior to this admission. He had been maintained on danazol, vitamin B12, omeprazole and low-dose prednisone. He presented with a 2-week history of right leg swelling and fever. On the day of admission, he complained of cough with whitish sputum. At the ER, he was alert, oriented, hypotensive, tachycardic, tachypneic and febrile. He had pale conjunctivae and crackles on left lung base. A tender, erythematous, fluctuant 5x8 cm mass was noted on his right anterior leg; the remainder of the examination was normal. Patient was pancytopenic. Culture of intraoperative wound aspirate grew *Salmonella* Typhi. Sputum culture grew *Pseudomonas aeruginosa*, *Enterobacteriaerogenes*, and methicillin-resistant *Staphylococcus epidermidis* (MRSE). Blood cultures grew *P. aeruginosa* and MRSE. Patient was treated successfully with surgical drainage and intravenous antibiotics (1-week piperacillin-tazobactam then 2-week ceftazidime and clindamycin with 1-week amikacin). He was discharged after 3 weeks with clinical recovery. As of this writing (4 months post-discharge), patient has stable disease with no new symptoms.

MDS predispose patients to severe *Salmonella* infections ranging from infected subdural empyema, aortitis, empyemathoracis to splenic abscesses. There has been no reported case of *Salmonella* soft tissue abscess in MDS. This is the first reported case of *Salmonella* (Typhi) soft tissue abscess in MDS. *Salmonella* infections should be considered in the differential diagnosis of soft tissue abscesses especially among immunocompromised patients such as MDS.
Does the use of fabric conditioners increase the proportion of antiseptic resistance gene positive staphylococci?

HY Chiu, MV Boost, MM O’Donoghue

Squina International Centre for Infection Control, School of Nursing, The Hong Kong Polytechnic University, Kowloon, Hong Kong.

Background

Fabric conditioners containing quaternary ammonium compounds (QACs) and other anionic and cationic compounds, are widely used in laundering of clothes. These compounds are also used as disinfecting agents. Increased resistance to QACs as well as to cationic biocides including chlorhexidine has been observed in staphylococcal isolates from clinical and food sources harbouring plasmids containing the qacA/B, smr, qacG, qacH and qacJ genes, which encode multidrug efflux pumps. This study investigated staphylococcal isolates from household washing machines for presence of QAC genes.

Method

Swabs were collected from the conditioner holder and the inner surface of 112 washing machines 15-30 minutes after completion of a washing cycle. Swabs were placed in 5% salt brain heart infusion broth and incubated overnight followed by sub-culture onto \( \text{SoSelect} \). After incubation, all staphylococci were tested for coagulase activity. Genes for antiseptic resistance were amplified by PCR and visualized on agarose gel.

Results

Staphylococci were isolated from 81 (72%) machines. \( S. \text{aureus} \) was present in 30 (26.8%) and CNS in 66 (58.9%), both being present in 15 and 16 machines yielding two species of CNS. Of the total 112 isolates, 54 (48%) carried a QAC gene of which 44% harboured qacA/B, 16% smr and 19% qacH. Neither qacG or qacJ were detected. Eighteen isolates carried two or more QAC genes. More staphylococci were isolated from the interior of the machines (N=76) than the fabric conditioner containers (N=26).

Conclusions

Both \( S. \text{aureus} \) and CNS were able to survive the washing cycles in a high proportion of machines despite the use of washing detergents. This may be more likely if a low temperature washing cycle is used. Of the staphylococci isolated, over half carried one or more QAC genes suggesting that there is selective pressure for development of increased tolerance to disinfecting agents in laundry products. More isolates were found on the interior surfaces suggesting that they originated from clothing, but survival in the presence of undiluted conditioner indicates tolerance to higher QAC concentrations. As there is evidence of co-selection for QAC genes and resistance determinants for several antibiotics, survival of these organisms may be a cause for concern.
Laboratory based algorithm in the evaluation of Acute Febrile Illness in Children

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1. Consultant Infectious Disease, Apollo Children Hospitals, Chennai, India.

Background: Acute febrile illness (AFI) are a major cause of morbidity in children who reside in or travel to tropical countries. Dengue, malaria, typhoid and scrub typhus were common etiologies of AFI in the developing world. Lack of guidelines on the initial approach to diagnosis & treatment of these infections results in unnecessary testing and inappropriate antimicrobial use in tropical countries. The objective of this study was to evaluate the role of basic laboratory tests in the diagnosis of common AFI in children.

Methods: We retrospectively analyzed medical & basic laboratory test records of children (age <16 years) admitted & diagnosed dengue (based on NS 1 & Ig M positivity), malaria (based on blood smear), typhoid (based on blood culture) and scrub typhus (using Ig M ELISA) in a tertiary care pediatric hospital in South India between 2012-2013 and evaluated their utility in final diagnosis.

Results: Of 153 cases reviewed, the common laboratory parameters analyzed were shown in table 1.

Table-1 Basic Laboratory features of common AFI in children

<table>
<thead>
<tr>
<th></th>
<th>Dengue</th>
<th>Malaria</th>
<th>Typhoid</th>
<th>Scrub typhus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of cases</td>
<td>68</td>
<td>39</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>HB (gm/dl) mean</td>
<td>12.48</td>
<td>10.05</td>
<td>11.25</td>
<td>10.28</td>
</tr>
<tr>
<td>WBC (cells/cumm) mean</td>
<td>6483</td>
<td>7291.8</td>
<td>7831.8</td>
<td>9527.2</td>
</tr>
<tr>
<td>Eosinopenia (Eosinophil count 0 or 1)</td>
<td>73.81%</td>
<td>53.84%</td>
<td>100%</td>
<td>72.72%</td>
</tr>
<tr>
<td>Platelets (mean)</td>
<td>65633.3</td>
<td>55,043</td>
<td>1,88,877</td>
<td>51,833.3</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.86</td>
<td>5.40</td>
<td>6.37</td>
<td>5.25</td>
</tr>
</tbody>
</table>

Normal hemoglobin, normal to low WBC counts and thrombocytopenia were associated with dengue. Low hemoglobin, normal WBC counts & thrombocytopenia were features of malaria, Eosinopenia along with normal blood counts were diagnostic of typhoid. Normal to high WBC counts & thrombocytopenia were suggestive of Scrub typhus in children. CRP is widely variable however, it is normal in dengue fever.

Conclusion: Judicious interpretation of common laboratory tests in children with AFI will help not only in diagnosing etiology of fever but also avoiding unnecessary tests & antibiotics misuse in tropical countries.
Association of virulence factors with phylogenetic groups of *Escherichia coli* isolated from UTI patients from Badin, Pakistan

N Kumar, F Nahid, R Zahra*

Department of Microbiology, Quaid-i-Azam University, Islamabad 45320, Pakistan

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**Results:** Thirty four isolates were assigned to group B2, while 23, 2, 1, 7 and 22 isolates belonged to groups F, B1, A/C, clade I/II and unknown, respectively. Prevalence of *papC* (79%) was highest followed by *aer* (60%), *papGII* (13%), *papGIII* (11%), *cnf* (8%), *hly* (4%) and *sfa* (1%). Three isolates were positive for *hra* gene.

**Conclusion:** This data provides the prevalence of phylogenetic groups and virulence associated genes in uropathogenic *E. coli* strains causing infection in Badin, Pakistan.
Patterns of Spread Leptospirosis Disease In Yogyakarta

Lilis Suryani*

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Abstract

Climate change impacts caused floods in Indonesia. High intensity by brief periods of rain caused flooding everywhere. As a result is increase in outbreaks of diarrheal diseases and leptospirosis, which usually happen after the flood. Leptospirosis is an acute fevers disease caused by Leptospira. The disease usually appears in rainy season, especially in the areas which became subscriptions flood. Leptospirosis is a zoonotic diseases transmitted to humans via rodents, dogs and some farm animals. Indonesia is the country with the device to third incidents of leptospirosis in the world with the death reaching 2.5% - 16.45%. Yogyakarta is the highest mortality rate leptospirosis with case fatality rate of 19.23 in 2011. The objectives of this research were to study the patterns of spread leptospirosis in Yogyakarta. The spread of leptospirosis varying from one place to another, so space or spatial components is part of influence in the process of scattering. This research had been done to spatial analyze the patterns spread of leptospirosis in Yogyakarta.

This research is observational design. Data patients with leptospirosis is taken from medical record in 2011-2013 from Wirosaban hospital, Yogyakarta city. Linkages in the spatial spread of the leptospirosis disease is measured through spatial autocorrelation using index Moran. The patterns of incidence leptospirosis is examined by Average Nearest Neighbour, while mapping to indicate areas that have a high degree to low of risk in the spread of leptospirosis performed using kernel density estimation.

The results of the study showed that the patterns of spread leptospirosis disease in Yogyakarta is clustered. There is no spatial autocorrelation in the leptospirosis spread. Kernel density estimation based on operating district has a high degree of risk in the spread of the disease leptospirosis in Yogyakarta. Umbulharjo is the high risk area of incidense leptospirosis.
DRUG RESISTANCE OF *STAPHYLOCOCCUS AUREUS* AND *S. EPIDERMIDIS* ISOLATED FROM HANDLES OF SHOPPING TROLLEYS IN SUPERMARKETS IN MALAYSIA

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**Background**

Interaction between microbes and humans can lead to infection. Contact can happen in a number of different ways. The microbe might directly contact the host, or it might contact the host via an indirect route involving inanimate objects (fomites). Numerous studies on the contamination of methicillin-resistant *Staphylococcus aureus* (MRSA) on objects in healthcare setting have been conducted and they are tourniquets, doctors and nurses’ pens, mattresses, television sets, faucet handles, over-bed tables, blood pressure cuffs, computers’ keyboards, room door handles, immersion bathtubs, chairs for shower and stretchers for bathroom. Contamination of methicillin-resistant coagulase-negative staphylococci and MRSA on touch surfaces in public transport have also been reported by other investigators in Belgrade, Portugal and Japan. In Malaysia, MRSA has been reported in hospital, among healthy carriers, pigs and pig handlers and doctors’ neckties. However, there are no report on contamination of MRSA and methicillin *Staphylococcus epidermidis* (MRSE) on the handles of shopping trolleys.

**Methods**

A total of 340 handles were swabbed to isolate antibiotics resistant *Staphylococcus aureus* and *S. epidermidis* from the handles of shopping trolleys, and the samples were used for bacteriological tests. A total of 15 antibiotics were tested on *S. aureus* and *S. epidermidis* isolates according to the procedures of Clinical and Laboratory Standards Institute (formerly NCCLS). The antibiotics tested were: Amoxicillin-clavulanic acid, Ampicillin-sulbactam, Ceftazidime, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Enrofloxacin, Flumequine, Gentamicin, Lincomycin, Methicillin, Sulfamethoxazole, Tetracycline, Trimethoprim and Vancomycin. Detection of mecA gene by polymerase chain reaction was conducted on methicillin-resistant *S. aureus* and *S. epidermidis*.

**Results**

A total of 11 of *S. aureus* and two *S. epidermidis* isolates were resistant to more than two antibiotics. The mecA gene was detected in two *S. aureus* and two *S. epidermidis* isolates.

**Conclusion**

This is the first report on isolation of drug resistant *S. aureus* and *S. epidermidis* from handles of shopping trolleys in Malaysia. The public health importance of these drug resistant staphylococci will be discussed.
Detection of anti-Leishmania antibodies among Asymptomatic Contacts of Patients with Kala-azar in Bangladesh

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1Centre for Infectious Diseases & Microbiology Laboratory Services, Westmead Hospital, Australia; 2The University of Sydney, Australia; 3Directorate General of Health Services, Bangladesh

Abstract

Visceral leishmaniasis is a vector borne and an emerging parasitic infection caused by an intracellular protozoan parasite of the genus Leishmania. It is primarily a zoonosis and is transmitted to humans and other vertebrate hosts by the bite of a female phlebotomine sand fly. Commonly known as kala-azar (KA), this infection poses a major public health problem in the centre and the north-western quarter of Bangladesh. The annual incidence of KA is about 40,000-45,000 cases with more than 40.6 million people at risk. About 300 cases per 10,000 populations per year are reported from the highly endemic district of Mymensingh bordering with the highest endemic Indian states of Bihar and West Bengal.

We carried out a cross sectional study among 257 asymptomatic and apparently healthy contacts of KA patients. These healthy subjects came with infected patients from different areas of Bangladesh attending Surya Kanta Kala-azar Research Centre (SKKRC), Mymensingh district, from May, 2013 to May, 2014. Among 257 study subjects 208 (80.93%) were family members and 49 (19.07%) were adjacent neighbours. Recombinant 39-amino acid-repeat antigen containing immunochromatographic test (rk39 ICT) and enzyme linked immunosorbent assay (ELISA) using crude promastigote antigen were done on plasma specimens of asymptomatic contacts to detect anti-Leishmania antibodies. The results show that 21.40% (55/257) were positive by either of the two tests. Of which 67.27% (37/55) were positive by rk39 ICT, 83.64% (46/55) by ELISA and 50.91% (28/55) by both of these tests. Among sero-positive individuals 49 (23.56%) were family members of the index cases and 6 (12.24%) were adjacent neighbours. Further confirmatory tests are underway but the study results so far show a higher number of asymptomatic human carriers who reside closely with infected patients. This indicates that diagnosis and treatment of asymptomatic human cases should be considered as part of the strategy in controlling human leishmaniasis.
Characteristics of extended spectrum β-lactamase producing Gram negative bacteria isolated from Mongolian patients

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Center of Infectious Disease and Signaling Research, College of Medicine, National Cheng Kung University, Tainan, Taiwan⁵

Keywords: β-lactam resistance, CTX-M, genetic relatedness

Background: Beta-lactam antimicrobial agents represent the first line treatment for bacterial infections and spread of ESBL among Gram-negative bacteria calls for concern, especially in view of treatment failure, high treatment cost, and consequent high morbidity and mortality of patients. The aim of this study was to characterize and to investigate the ESBL-producing bacteria isolated Mongolian patients in 2013.

Methods: A total of 185 Gram negative bacteria were collected from the patients of two tertiary care hospitals. Genotypic and phenotypic methods were used to characterize ESBLs. PFGE was used to determine genetic relatedness of isolates. Detection of CTX-M-like ESBLs was carried out using PCR with primers specific for the CTX-M, TEM, and SHV types of ESBLs.

Results: 25 out of 185 strains were positive for ESBLs by phenotypic methods. The CTX-M gene was highly prevalent among the ESBL-positive E. coli strains (n=19). The CTX-M15 (n=9), CTX-M14 (n=6), CTX-M27 (n=1), CTX-M24 (n=1), CTX-M55 (n=3) and CTX-M15/CTX-M14 (n=1) gene was detected in E.coli strains. Also K.pneumoniae isolates were found to harbor CTX-M3, CTX-M22 together with SHV-1 and TEM-1. PFGE analysis showed that the three CTX-M type of ESBL producing K. pneumoniae strains were clonal and spread in one hospital.

Conclusions: This study showed high prevalence of ESBLs in E.coli isolates, with CTX-M-15 as the predominant ESBL gene. Our study demonstrates possible outbreaks of CTX-M producers in Mongolian hospitals. This study represents the first report of CTX-M-22, CTX-M-27 and CTX-M-55 β-lactamases E.coli isolates in this area.
Background
The term anaerobe is referred to an organism that requires reduced oxygen for growth. Anaerobic organisms are major component of normal flora in the human mucous membrane. These organisms generally cause disease subsequent to the breakdown of mucosal barriers & allowing the bacteria to enter the sterile sites. Anaerobe organisms cause bacteraemia for 10-20% of cases and yet mortality associated with anaerobic bacteraemia often among highest. Risk factors for anaerobic infection include immunocompromised state, chronic illnesses and prolonged usage of wide spectrum antibiotics.

Methodology
The anaerobe isolates and their antibiotics sensitivity from clinical specimens in Pathology Department Sungai Buloh Hospital were identified retrospectively from the records. All isolates from the year 2010 to 2013 were included.

Results
From the year 2010 to 2013, total anaerobe organisms isolated from clinical specimens in Sungai Buloh Hospital were 276. The highest isolate was in year 2012 where total isolates is 125. The most frequently isolated anaerobe was Clostridium difficile (n = 42, 15.2%) from stool specimens followed by Clostridium spp. (n = 34, 12.3%) and Bacteroides fragilis (n = 29, 10.5%). Anaerobe organisms were mostly isolated from blood culture where the totals of 139 blood cultures were positive for anaerobes. Bacteroides fragilis was the highest anaerobe organisms isolated from the blood culture (n = 22, 15.8%). Sensitivity pattern on year 2010 showed some of the anaerobes organisms that resistant towards imipenem which include Bacteroides fragilis, Peptostreptococcus spp., Eggertella spp. and Prevotella oralis. One Clostridium spp. was resistant to vancomycin and one Peptostreptococcus spp. was resistant towards metronidazole. On 2013, the sensitivity pattern showed most organisms isolated were sensitive towards antibiotics tested.

Conclusion
Bacteraemia caused by anaerobic organisms is the commonest infection compared to other anaerobic bacterial infections in Sungai Buloh Hospital.
Outpatient Parenteral Antibiotic Therapy for Bone and Joint Infections in a Singapore Restructured Hospital

L Lum, B Alferez, Z Sulaiman, D Fisher, N Chew

National University Health System, Singapore

Introduction: Bone and joint infections (BJI) constitute a significant proportion of patients seeking treatment in the Outpatient Parenteral Antibiotic Therapy (OPAT) center in the National University Hospital (NUH) Singapore.

Objectives: To describe treatment outcomes for patients with BJI who were treated at the NUH OPAT center between January 2006 and December 2012.

Methods: A prospectively maintained registry of all patients treated was examined for cases of BJI. Data on patient demographics (including age, gender, and ethnicity), co-morbidities (including diabetes mellitus, prosthetic joint or foreign device), history of open fractures, microbiological findings (including quality of culture sample, microorganism and presence of MRSA and multidrug resistant gram negative bacteria) and total duration of antibiotic treatment were collected. We examined for sustained BJI treatment success at (i) 0-6 months and (ii) 6-12 months after completion of OPAT antibiotic therapy.

Results: Two hundred and forty cases of BJI were identified and treated through OPAT. Overall treatment success rate was 85.4%. 35 (14.6%) patients had a relapse of BJI within 6 months of completion of OPAT; 6 patients (2.5%) had a recurrence of BJI between 6 and 12 months after completion of OPAT. On multivariate logistic regression analysis, relapse of BJI within 6 months of OPAT completion was associated with male gender (p=0.006) and inadequate tissue biopsy for microbiology culture (p=0.03). More patients who received ≤6 weeks of antibiotics relapsed compared to those who received >6 week antibiotic treatment, although not statistically significant (18% vs 12%; p=0.07). Recurrence of BJI between 6 to 12 months after OPAT completion was associated with MRSA infection (p=0.02).

Conclusion: For the majority of patients, BJI can be successfully managed through OPAT. Most BJI relapses occurred within 6 months of OPAT completion. Identification of those more likely to have a BJI relapse, including male patients and those with inadequate microbiology culture may encourage enhanced and prolonged surveillance in these subgroups of patients.
Fosfomycin is an independent cause of hypernatremia in ICU patients

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Chi-Mei Medical Center, Tainan, Taiwan.

Background
The increased usage of an old antibiotic fosfomycin was noted for the treatment of emergent drug-resistant Enterobacteriaceae. Hypernatremia has been a concern with this sodium-containing (14.5mEq/g) antibiotic. The aim of this retrospective study is to elaborate the problem of fosfomycin-associated hypernatremia.

Methods
Patients that have been using fosfomycin for more than one day during their admission in ICU were identified for the period of June 2011 to March 2012 in a 92-bed ICU of a 1200-bed tertiary hospital. Demographic data, underlying disease, dosage and duration of fosfomycin consumption, daily fluid intake and output, serum sodium level were retrieved from the electronic medical records.

Results
Hypernatremia (> 145mEq/L) were noted in 78 out of 138 patients (56.5%) with fosfomycin treatment. Multivariate logistic regression showed that initial hypernatremia, total dose of fosfomycin used and hydrocortisone used were independent risk factors for hypernatremia. While hemodialysis was a protecting factor. The sodium increased 1.54 ± 1.68 mEq/L for each day of fosfomycin used and increased 0.50 ± 0.56 mEq/L for each dose of fosfomycin used.

Conclusion
Hypernatremia is very common among fosfomycin users, especially with concomitant use of hydrocortisone and diuretics, but less frequent in patients underwent hemodialysis. Total fosfomycin used is an independent risk factor for hypernatremia after correcting other possible confounding factors.
The Ability of biofilms formation And the Differences of Antifungal susceptibility pattern between clinical isolates of planktonic Candida sp and biofilm Candida sp

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Abstract

Background: Candida sp is known as a normal flora in humans’ body. However, it may cause many kind of infection worldwide. Manifestation of Candida sp infections are associated with the ability to form biofilms. A cell covered by biofilm is unreachable by the host immune responses and the effects of antifungal. Consequently, the infection is difficult to be treated with conventional therapy. Biofilms of Candida sp show resistance to broad-spectrum antifungal. This study were to determine the antifungal susceptibility pattern of Ketoconazole, Itraconazole and Fluconazole against Candida sp clinical isolates which form biofilms and to identify the difference of antifungal susceptibility pattern between clinical isolates of biofilm Candida sp and planktonic Candida sp.

Method: Laboratory descriptive method with cross sectional design using clinical isolates of Candida sp collected from the Laboratory of Microbiology Faculty of Medicine Gadjah Mada University Yogyakarta. The ability to form biofilm was tested on 96-wheel microplate and the intensity of biofilm formation was determined by staining with Crystal Violet. The antifungal susceptibility test of clinical isolates of both planktonic and biofilm Candida sp was performed by using liquid dilution method on 96-wheel microplate.

Results: Among 24 clinical isolates of Candida sp, 70.8% has strong intensity on the ability to form biofilm and 29.2% showed moderate intensity. Susceptibility pattern of clinical isolates of planktonic Candida sp against Fluconazole, Ketoconazole and Itraconazole are 100%, 78.9% and 57.9%, respectively. Meanwhile the susceptibility pattern of clinical isolates of biofilm Candida sp against Ketoconazole, Itraconazole and Fluconazole are 31.6%, 21.1% and 31.6% respectively. This study indicates a decline on percentage of sensitive and an increase on percentage of resistant among clinical isolates of Candida sp. The MIC values of clinical isolates of biofilm Candida sp was 2 to ≥ 33 times more than the MIC values of clinical isolates of planktonic Candida sp.

Conclusion: The difference on the antifungal susceptibility between clinical isolates of planktonic Candida sp and clinical isolates of biofilms Candida sp were decline pattern of the sensitivity percentage and an increase pattern of resistance percentage. MIC values of clinical isolates of biofilm Candida sp were 2 to ≥ 33 times higher than the MIC clinical isolates of planktonic Candida sp.

Keywords: Biofilm Candida sp, Antifungal susceptibility pattern, MIC values
The detection of adherence factors by *Escherichia coli* cause of urinary tract infections in Ulaanbaatar, Mongolia


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**Background:** Urinary tract infection is among the most common bacterial infectious diseases encountered at all ages. *Escherichia coli* is being the etiologic agent in 50–80%. Therefore, it is an important public health problem. *E.coli* causing urinary tract infections express pili, fimbriae and others adherence virulence factors. In this study we detected the some adherence virulence factors of *Uropathogenic Escherichia coli (UPEC)* in Ulaanbaatar, Mongolia.

**Methods:** A total of 76 *E.coli* samples were collected. These samples were positive bacteriological examination of urine, performed at the bacteriological laboratory of the State Central Third Hospital and State Central First Hospital, Ulaanbaatar, Mongolia. The biofilm formation was evaluated by the growth rate of *E.coli* on plastic surface. The detection of the virulence factors type 1 fimbriae (*fimA* gene) and P-fimbriae (*papC*) was performed by multiplex PCR using gene specific primers. Curli expression was determined by using Congo red agar.

**Results:** The evaluation of bacterial biofilm formation using 96 well plates showed 40 negative (52.6%), 32 weak biofilm (42.1%) and 4 moderate biofilm (5.3%) formation for *E.coli* and no strong biofilm forming strain was detected. The cell surface protein (curli) was detected by Congo red agar. The result was 71% positive for studied *E.coli* strains. The detection result of pili genes by multiplex PCR showed that *fimH* gene detected for 73 (96.1%) and *papC* gene detected for 18 (23.7%) *E.coli* cultures.

**Conclusion:** Almost half of surveyed Uropathogenic *E.coli* isolated in Ulaanbaatar, Mongolia had ability of biofilm formation and it has been determined by the bacterial surface protein (curli), which is one of bacterial adherence factors, may cause biofilm formation.
Detection of Anti-\textit{Leishmania} Antibodies among Asymptomatic Contacts of Patients with Visceral Leishmaniasis in Bangladesh

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Abstract

Visceral leishmaniasis (VL) is a vector borne and an emerging parasitic infection caused by an intracellular protozoan parasite of the genus \textit{Leishmania}. It is primarily a zoonosis and is transmitted to humans and other vertebrate hosts by the bite of a female phlebotomine sand fly. Commonly known as kala-azar, this infection poses a major public health problem in the centre and the north-western quarter of Bangladesh. The annual incidence of VL is about 40,000-45,000 cases with more than 40.6 million people at risk. About 300 cases per 10,000 populations per year are reported from the highly endemic district of Mymensingh bordering with the highest endemic Indian states of Bihar and West Bengal. Asymptomatic carriers of VL are difficult to detect and these individuals could aid in the spread of this infection in a community. The aim of this study was to determine the level of asymptomatic infection present in Bangladesh. We carried out a cross sectional study among 257 asymptomatic and apparently healthy contacts of VL patients. These healthy subjects came with infected patients from different areas of Bangladesh attending Surya Kanta Kala-azar Research Centre (SKKRC), Mymensingh district, from May, 2013 to May, 2014. Among 257 study subjects 208 (80.93%) were family members and 49 (19.07%) were adjacent neighbours. The immunochromatographic test containing recombinant antigen (rk39 ICT) and enzyme linked immunosorbent assay (ELISA) using crude promastigote antigen were done on plasma specimens of asymptomatic contacts to detect anti-\textit{Leishmania} antibodies. The results show that 21.40% (55/257) were serologically positive. Of which 67.27% (37/55) were positive by rk39 ICT, 83.64% (46/55) by ELISA and 50.91% (28/55) by both of these tests. Among sero-positive individuals, 49 (23.56%) were family members of the index cases and 6 (12.24%) were adjacent neighbours. Further confirmatory tests are underway, but the study results so far show that a higher number of asymptomatic human carriers reside closely with infected patients. This indicates that diagnosis and treatment of asymptomatic human cases should be included as part of the strategy in controlling VL.
ESBL genes and phenotypic activity of Klebsiella pneumoniae in a Malaysian district hospital

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Background: Extended-Spectrum Beta-Lactamase (ESBL) is one of the enzymes that are routinely screened in the clinical laboratory. This enzyme is consistently associated with multiple drug resistant organisms. It can be produced by a variety of bacteria genus including Enterobacteriaceae group, Pseudomonas spp, Acinetobacter spp. and many more. Dissemination of ESBL genes across bacteria species via plasmid can result in high incidence of antibiotic resistance. ESBL distribution has been reported from all over the world including South East Asia. In some countries especially in Europe, the distribution pattern now is shifting from one dominant ESBL enzyme to a new emerging group of ESBL. There is still minimal data reported in Malaysia, particularly from district hospitals. The aim of this study was to identify the association between phenotypic activity of ESBL enzymes and ESBL genes by polymerase chain reaction (simplex PCR).

Methods: Beta-lactam resistant Klebsiella pneumoniae isolates were collected from various clinical specimens in Hospital Muar, Malaysia (2009, 2012). Antimicrobial susceptibility pattern was determined and ESBL enzyme was detected by disk diffusion method using the Clinical and Laboratory Standards Institute (CLSI 2012) guideline. All isolates were also phenotypically tested for production of carbapenemase and AmpC beta-lactamase by Modified Hodge test and cloxacillin impregnated Muller Hinton agar respectively. ESBL determinant genes (blaTEM, blashv, blaCTX-M-1, blaCTX-M-2, blaCTX-M-8, blaCTX-M-9 and blaCTX-M-25) were identified using simplex PCR. Results: Of the 141 isolates, 71.6% showed ESBL activity, either alone (51.8%) or in combination with carbapenemases and/or AmpC beta-lactamase enzymes (19.9%). One hundred and twenty one (85.8%) isolates harbored ESBL genes with blashv being the predominant gene detected (75.2%), followed by blaCTX-M-1 (44%), blatEM (41.1%) and blaCTX-M-9 (0.7%). More than half carried multiple ESBL genes and a combination of blashv, blaCTX-M-1 and blatEM was the most frequent. There was an association between Klebsiella pneumoniae-ESBL genes and phenotypic activity. Twenty two isolates that showed negative ESBL activity harbored ESBL genes. Antibiotic susceptibility testing showed high resistance rate to antibiotics ranging from 45-80% for aminoglycosides, 80-90% for cephalosporins to more than 90% for beta lactams/beta lactamase inhibitor and penicillin group. Carbapenem resistance for ertapenem, meropenem & imipenem were 60%, 74% and 85% respectively. Conclusion: Detection of ESBL was high among Klebsiella pneumonia isolates. Among the ESBL producers, blashv was the most frequent gene detected and majority of them carried multiple ESBL genes. High carbapenem resistance is alarming and need further investigation.
Tuberculosis infection among HIV/AIDS patients: the Philippine General Hospital STD/AIDS Guidance Intervention Prevention Unit (PGH SAGIP Unit) experience

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Background: HIV is the greatest risk factor for developing tuberculosis (TB). In the Philippines, studies on HIV-TB coinfection are limited. This study aimed to determine the prevalence and describe the clinical profile of tuberculosis among HIV/AIDS patients managed at PGH SAGIP Unit.

Methods: After institutional and ethical approval of the research protocol, 352 coded records of HIV/AIDS patients managed at PGH SAGIP Unit from 1995 till February 2011 were reviewed. PGH SAGIP Unit is an outpatient clinic set in a tertiary hospital for the prevention, diagnosis and treatment of HIV/AIDS patients. Pulmonary TB (PTB) was defined as a chest X-ray result consistent with TB, smear-positive for AFB, culture-positive, or PCR-positive for \textit{M. tuberculosis}. Extrapulmonary TB was defined as biopsy-proven, tissue positive for AFB, or culture-positive for \textit{M. tuberculosis}.

Results: Out of the 352 HIV/AIDS patients, 39 had tuberculosis (34 males). Median age of HIV-TB coinfection was 32. Tuberculosis (9.7%) was the most common presenting opportunistic organisms at first consult. Of the 39 HIV-TB cases, TB infection at first consult, at 1 year, and at 5 years were 31, 7, and zero new cases, respectively. Pulmonary involvement was 82.1% followed by the lymph nodes (20.5%) and the gastrointestinal tract (5.1%). Three patients had both lung and lymph node involvement. Weight loss and cough were present in 66.7% and 64.1%, respectively. Among the 30 patients with chest X-ray, 53.3% had upper lung infiltrate followed by lower lobe infiltrates (13.3%) and cavitation (10%). Normal chest X-ray was present in 20%. One had isoniazid resistance. Another had resistance to isoniazid, rifampicin, pyrazinamide, and ethambutol. Mycobacterium other than tuberculosis (MOTT)-PTB coinfection was present in one patient. Only 25 of the 32 patients with PTB had sputum AFB done with a 48% positivity rate. CD4 count less than 50 was seen in 39.4%. Among HIV-TB patients on active follow-up (24 of 39), ARVs were ongoing in 20 patients.

Conclusion: TB was the most common presenting opportunistic infection. TB presented most commonly at 1st consult. The lungs were the most common site of tuberculosis followed by the lymph nodes. Weight loss and cough were the two most common symptoms. Upper lung infiltrate was the most common finding on chest X-ray. CD4 counts were frequently below 50. This study recommends a computerized registry system for patient records for quick retrieval of patient information to be used for patient care and future research. Local, larger prospective studies on prevalence, prevention, and treatment HIV/TB coinfection should be done. The study was limited to the completeness and availability of records for review.
Leptospirosis is an emerging zoonosis caused by pathogenic spirochetes belonging to the genus Leptospira. Early diagnosis is essential because antibiotic treatment is most effective when initiated early in the course of the disease. Culture and microscopic agglutination tests are gold standard methods for diagnosis of the disease; however, they are laborious and time consuming. Detection of leptospiral DNA by PCR has been demonstrated to be useful for specific and early diagnosis of leptospirosis. In the present study, efficacy of a specific set of primers targeting a specific sequence of outer membrane protein gene (OMP) i.e. lipL32 gene, was tested using serum samples from patients clinically suspected of leptospirosis, as well as standard reference serovars of Leptospira interrogans and environmental isolates of Leptospira. This gene was targeted as it was reported to be conserved and present in all pathogenic Leptospira serovars. A pair of specific primers flanking approximately a 789 bp of lipL32 gene of leptospire was designed using the primer BLAST program. A total of 132 anonymous serum samples suspected of leptospirosis were tested by PCR using the primers. They were obtained from the Leptospira Diagnostic Laboratory, Bacteriology Unit of IMR. Reference pathogenic leptospiral serovars namely Leptospira interrogans serovars Australis, Autumnalis, Bataviae, Djasiman, Hebdomanis, Javanica, Pomona, Pyrogenes, Tarassovi and Sedjro and ten (10) environmental isolates of Leptospira were also tested by the PCR. The lower limit of detection of the PCR was determined by performing the amplification on known negative serum sample spiked with culture of Leptospira serovar Patoc followed by serial dilution. DNA sequencing was carried out on the PCR products to confirm the gene. Positive amplification resulted in PCR products of about 788 bp as detected by the agarose gel electrophoresis. All of the 10 reference pathogenic Leptospira serovars gave positive PCR results. Out of 132 serum samples tested, 83 (62.8%) yielded positive amplification. As for the environmental leptospire isolates, none was positive by the PCR. DNA sequencing of the PCR products revealed 99-100% identity with the L. interrogans lipL32 gene. The lower limit of detection of the PCR achieved was at the 1:10^6 dilution of the cultured Leptospira. Preliminarily, results of the study indicated that PCR detection of lipL32 gene could be used for diagnosis of leptospirosis, and owing to its rapid nature is potentially useful for early diagnosis. Further study is needed to validate the findings and to determine the specificity and sensitivity of the PCR on a large number of samples.
Antibiotics in surgical wards – use or misuse? A developing country’s perspective

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Background
Appropriate use of antibiotics in surgery has been associated with lower risk of postoperative infection and reduced risk of development of antimicrobial resistances. However, antibiotic usage in surgical setting remains variable and haphazard. There are limited studies that have explored the appropriateness of therapeutic antibiotic use among surgical patients and few studies have focused on the impact of an antimicrobial stewardship program (ASP) in surgical patients, particularly in a developing country. Therefore, this study evaluated the appropriateness of antibiotics prescribed among surgical patients in a developing country.

Methods
A prospective observational study was conducted in surgical wards at a tertiary teaching hospital in Malaysia. The appropriateness of antibiotic therapy was based on a review by an expert panel (which consisted of two infectious disease consultants and a pharmacist), and compliance with either the Malaysian National Antibiotic Guidelines 2008 or international clinical practice guidelines on antimicrobial prophylaxis in surgery.

Results
A total of 129 patients who were prescribed antibiotics were included in the study. On average, each patient was prescribed 4.8±3.6 antibiotics. Out of 593 antimicrobial regimes prescribed for these surgical patients, the prevalence of inappropriate use of prophylactic and therapeutic antibiotics was 66.3% and 42% respectively. Ninety-five (73.6%) of the patients had received at least one course of inappropriate antimicrobial. Prophylactic antibiotics were more frequently inappropriate than therapeutic antibiotics (P< 0.001). Inappropriate prophylactic antibiotics most commonly occurred due to inappropriate timing (36.4%) and inappropriate duration of therapy (34.5%). The most common reasons for inappropriateness in therapeutic antibiotics were inappropriate choice of antibiotic (42.1%) and inappropriate indication for antibiotic use (40.7%). Aminoglycosides (66.7%), second generation cephalosporins (60%) and carbapenem (56.1%) were classes of antibiotics which had the highest prevalence of inappropriate therapeutic use. Inappropriate antibiotic use was not found to have significant impact on hospitalisation days (P = 0.670) and all-cause mortality (P = 0.727). However, the high readmission rate due to relapse of infection (32% of readmission) and high prevalence of sepsis as a reported cause of death are of concern. Although, we were unable to ascertain the direct role that inappropriate antibiotic use plays in these occurrences but at the very least, it would be prudent to optimize the use of antibiotics.

Conclusion
Our study suggests considerable misuse of both prophylactic and therapeutic antibiotics in the surgical wards; and provides a pivotal guide for antimicrobial stewardship initiatives.
Is tigecycline prescribed to treat carbapenem-resistant *Acinetobacter baumannii* complicated urinary tract infections

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**Background**
In general, carbapenem-resistant *Acinetobacter baumannii* (CRAB) is resistant most of antibiotics, except tigecycline and colistin. Colistin has nephrotoxicity, so it is inappropriate antibiotic in some conditions, such as in patients with renal impairment. Tigecycline has low urinary concentration, so it is not usually recommended to treat urinary tract infections. Hence, in case of CRAB complicated urinary tract infections (cUTI), the treatment options are severe limited. In this study, we reported two cases of CRAB cUTI successfully treated with two different dose of tigecycline.

**Methods**
We reported two cases of CRAB cUTI treated with tigecycline 50 mg intravenous 12 hours per day for 14 days and 100 mg intravenous 12 hours per day (q12h) for 14 days, respectively. Standard disk diffusion method was used for antimicrobial susceptibility testing. The interpretation of tigecycline against *A. baumannii* isolates was according to the U.S. Food and Drug Administration interpretive criteria for Enterobacteriaceae (susceptible, minimal inhibitory concentration $\leq 2 \mu g/ml$). All intermediate results were regarded as resistant in this study.

**Results**
The two *A. baumannii* isolates were only susceptible to tigecycline (colistin was not tested). The two cases of CRAB cUTI treated with tigecycline were clinical treatment success; however, both repeated urine cultures were still positive with CRAB. Especially, in that case treated with tigecycline 50 mg q12h, CRAB was resistant to tigecycline in repeated urine culture.

**Conclusion**
As the above mentioned, the treatment options for CRAB cUTI are severe limited. In our option, tigecycline may be prescribed to treat CRAB cUTI, especially in case of no other appropriate antibiotics available. The reason of clinical treatment success may be tigecycline has good renal concentration. In contrast, the reason of being unable to eradicate CRAB, even select tigecycline-resistant CRAB isolate, should be it has low urinary concentration.
A case of multiple abdominal abscesses due to melioidosis: First case reported from North Sumatera, Indonesia
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Abstract

Background: Melioidosis is regarded as endemic in Southeast Asia and northern Australia. Unfortunately, published case reports and series are likely to represent only “tip of iceberg”, because culture facilities are not available in most endemic areas.

Case: MR, 13 yo boy, came to hospital with a 2-month history of abscesses and swelling in the abdomen and back. Subfebrile fever, dry cough and decrease body weight also found. He was hospitalized in orthopedic ward with diagnosis of suspected tuberculous spondylitis and received anti-tuberculosis drugs and abscesses drainage. Unfortunately, the abscesses recurrent. Screened for Tb was negative. He was referred to Pediatric Infectious Diseases ward after the result of pus culture using vitek found to be Burkholderia pseudomallei. Result of ultrasonography and CT-scan found multiple cystic and nodules in liver and spleen. The patient was treated with ceftazidime injection for 3 weeks and the symptoms were improved. He was discharged and continued with oral trimethoprim-sulfamethoxazole for 20 weeks.

Conclusion: This is the first reported case of meliodosis in a child in North Sumatera, Indonesia. The diagnosis of melioidosis was found from the result of pus culture using vitek. Patient was treated with Ceftizidime and continued with trimethoprim-sulfamethoxazole and the result was impressive.
Acquired metallo-β-lactamases and their genetic supports in clinical isolates of Gram-negative bacilli

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Background
Metallo-β-lactamases (MBLs) can hydrolyze almost all β-lactam antibiotics including carbapenems and are resistant to clinically available β-lactamase inhibitors. Numerous types of acquired MBLs have been identified, including IMP, VIM, NDM, SPM, GIM, SIM, KHM, DIM, TMB, PIM and AIM. IMP- and VIM-type MBLs are the most frequent MBLs and disseminate in members of the family Enterobacteriaceae, Pseudomonas spp., and Acinetobacter spp. Integrons are genetic elements capable of integrating gene cassettes by a site-specific recombination. Acquired MBL genes are often embedded in class 1 integrons and some are associated with ISCR elements. The integrons and ISCR are usually harbored in transposons and/or plasmids, forming so-called mobile vesicles for horizontal transfer of captured genes among bacteria.

Methods
Carbapenem-resistant clinical isolates of Enterobacteriaceae (Serratia marcescens, Klebsiella pneumoniae, Enterobacter cloacae) and Pseudomonas aeruginosa were collected from two distantly separated university hospitals during 1996 and 2013. MBL-producing strains were screened by a double-disk synergy test using ceftazidime and sodium mecaptoacetate (specific MBL inhibitor). Genes were determined by DNA sequencing after PCR amplifications.

Results
Co-expression of the blaIMP-1 and aac(6')Ile was observed within the identified intI1-blaIMP-1-aac(6')Ile-qacEΔ1 gene complex in clinical isolates of S. marcescens, K. pneumoniae and E. cloacae. The resistance genes were transferable from the wild-type bacterial hosts to E. coli HB 101 by conjugation, indicating that this gene complex is located on a conjugal plasmid. The S. marcescens strains were collected from inpatients at Showa University Hospitals (Tokyo) during 1996 and 2002, while the K. pneumoniae and E. cloacae strains were from inpatients at Showa University Yokohama Hospital (Yokohama City) after 2007, demonstrating the colonization of this plasmid-mediated integron-borne resistance gene complex in Enterobacteriaceae in the clinical setting. Additionally, IMP-1, IMP-7, IMP-10 and VIM-2 genes were detected in carbapenem-resistant clinical isolates of P. aeruginosa. All of these MBL genes were embedded in class 1 integrons and followed by one or two resistance genes encoding aminoglycoside-modifying enzymes, such as aac(6')Iae, aac(6')IIC, aacA7, aacC4, aadA1, aadA2 and aadA6.

Conclusion
By surveying the spread and colonization of resistance gene complexes in clinical important pathogens, we found that high prevalence of blaIMP genes harbored by class 1 integron has occurred in clinical isolates of Enterobacteriaceae and P. aeruginosa, which highlights the importance of molecular surveillance of such resistant genes.
Antibiotic resistance in invasive *Escherichia coli* studied in the years 2009-2012

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**Summary**

**Background:** The beta-lactamases with extended spectrum of activity (ESBL) are medically one of the most important group of enzymes. The another group of beta-lactamases representing of *Enterobacteriaceae* is group of the AmpC-type cephalosporinases that hydrolyzes all of beta-lactam antibiotics except carbapenems and unlike ESBL is poorly inhibited by clavulanic acid. The presented study provides identification and determination of the spectrum of resistance against different and clinically used antimicrobial drugs in the clinical isolates of invasive *Escherichia coli*.

**Methods:** These isolates had origin in different departments of the University Hospital L. Pasteur in Košice. The second goal was the detection of beta-lactamase production with extended-spectrum effect (ESBL) and testing of AmpC-type cephalosporinases by several phenotypic tests in clinical isolates. MALDI-TOF MS analysis was performed on a Microflex MALDI Biotyper (Bruker Daltonik) according to a standard sample preparation protocol of Bruker Daltonik. We used both microdilution method and method with the only active agent, respectively. Samples were positively tested for ESBL with the use of the CLSI disk diffusion method. PCRs were performed with a series of primers designed for the detection of Ambler class A, B and C beta-lactamase genes.

**Results:** That was investigated 307 strains of *E. coli*. The growth of *E. coli* resistance to selected antibiotic was present in 83,25 % of clinical isolates. There were identified 85 positive isolates in the studied group and the prevalence of the ESBL positive strains of *E. coli* reached 27,78 %. We used DDST and CLSI method for determination of the production of AmpC-type cephalosporinases and we detected three positive clinical isolates. One of the isolates produced both of ESBL and AmpC. Among AmpC beta-lactamases, the CIT and DHA types were detected. An *E. coli* strain was isolated with mutations in the promoter region of the AmpC chromosomal gene that are associated with overproduction of the relevant enzyme.

**Conclusions:** We describe a complex ESBL epidemiology. The study revealed a high rate of ESBL-producing *E. coli* isolates. *bla*TEM and *bla*SHV enzymes dominated in ESBL-positive *E. coli* isolates the University Hospital L. Pasteur in Košice.

**Keywords:** resistance, ESBL, β-lactamase, AmpC, prevalence
Antibiotic use and emerging of multidrug-resistant organism in intensive care unit: an observational study.
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Abstract

Background

The enormous antibiotic consumption is related with high prevalence of nosocomial infections in critically ill ICU patients. Besides its impact to healthcare cost, there is a possibility for antibiotic resistance induction and emergence multidrug-resistant pathogen. Surveillance studies such as infections incidence, antibiotic use, and antimicrobial susceptibilities of pathogens supply vital information regarding infection control and prevention of antibiotic resistance. There is scarcity of these data in our country. The result of this observational study may contribute as baseline for antibiotic stewardship program in the hospital.

Method

This study was designed as a retrospective study. The data was collected from medical record of patients hospitalized on 1st May up to 31st July 2013. This research was conducted at the general ICU M. Djamil Hospital, West Sumatera, Indonesia. In order to analyze antibiotic use, the data was taken from patients daily medication record during three month. We also analyze the susceptibility testing data from all patients. The data were analyzed by SPSS version 16.

Results

Of 82 patients admitted to ICU during the study period, 86.6% (71 patients) got at least one systemic antibiotic. Of all antibiotic, 85% were prescribed for surgical patients, only 15% for non-surgical condition. Most prescribed antibiotics were meropenem (26%), followed by metronidazole (24.5%), ceftriaxone (17.6%) and gentamycin (16.8%). About 52% patients received antibiotic combination with ceftriaxone-metronidazole as the majority combination (25%) while metronidazole occurred in 50% of all combination. Of all patients receiving antibiotics, only 28.2% (20/71) patients were checked for susceptibility of the pathogen. Most organism found on susceptibility test was Klebsiella spp. (59.1%), followed by E.coli (18.2%), Pseudomonas (13.6%) and S.aureus (9.1%). All organism found were multidrug resistant (MDR), while pandrug-resistant organism (PDR) was found in 9 out of 13 patients (69.2%) infected by Klebsiella.

Conclusion

We conclude that most antibiotics prescribed were for surgical patients. Most of therapy used carbapenem and metronidazole. Metronidazole was prescribed extensively as combination with other antibiotics, although anaerob bacteria infection were not proven. More than 50% patients received antibiotic combination. Gram negative organism is the major cause of infection in ICU patients. The emergence of multidrug-resistant organism in ICU has been showed by microbiology data.
Abstract

THE ROLE OF recA GENE PROTECTS AGAINST OXIDATIVE DAMAGE IN ENTEROCOCCUS FAECALIS

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Enterococcus faecalis is one of the aetiological agents of bacteremia, surgical wound infections, urinary tract infections, and endocarditis. In order to survive and disseminate, E. faecalis must be able to adapt to multiple environments and stresses both within and outside the host by altering its metabolic processes. One of the strategies is the SOS response that respond to DNA damage by inducing proteins for DNA repair such as the RecA protein. The ability of this protein to confer protection against oxidative damage has been demonstrated in a few organisms such as Escherichia coli, Salmonella enterica serovar Typhimurium, and Lactococcus lactis. In this study, the function of E. faecalis RecA was examined by comparison between the recA mutant and wild-type phenotypes. Alteration of this single gene had a pleiotropic effect, showing the involvement of RecA in cellular protection against stresses induced by oxidative agents. We also show that recA inactivation impairs the ability of E. faecalis to survive inside macrophages and to form biofilm. It is important to note that due to the limited therapeutic options available and cost of treating infections caused by enterococci, the prevention and spread of these bacteria is a major healthcare issue. Thus, understanding the physiology of E. faecalis may contribute to novel drugs that target metabolic pathways.
Antimicrobial properties of *Etlingerasessilanthera*

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In recent years, bacteria have become increasingly resistant to many conventional antimicrobials. Therefore, there is a need to search for new antimicrobials to overcome this issue. Tropical ginger from the genus *Etlingera* has been traditionally used amongst the natives of Sabah as medicine. This research evaluated the antimicrobial properties of the leaves, stems and roots of *Etlingerasessilanthera*. Crude extracts of the various parts of the plant were obtained with sequential solvent extraction, starting from hexane and followed by dichloromethane, ethyl acetate, methanol and water. Broth microdilution was then performed to determine the minimum inhibitory concentration (MIC) of the crude extracts against 18 strains of bacteria and a yeast. The hexane extract of the roots (R-HEX) yielded the lowest MIC against *Bacillus cereus* ATCC14579 (0.125 mg/mL) and was subsequently further studied. The mechanism of action was investigated through scanning electron microscopy (SEM) and membrane damage assay. These tests revealed that the cell membrane of *B. cereus* ATCC 14579 cells was damaged, resulting in a 90% reduction of viable cells when treated with R-HEX. Phytochemical screening revealed the presence of terpenoids in this fraction, suggesting that the bioactive compound might be a steroidal compound. In conclusion, this study showed that the hexane fraction of the roots of *E. sessilanthera* has promising antimicrobial activity. Further study is needed to purify and identify the compound that confers the antimicrobial property.
*Pasteurella multocida* pneumonia with associated bacteraemia: a retrospective review of cases from 1998 to 2014

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**Background**

*Pasteurella* are small Gram-negative coccobacilli. *Pasteurella multocida* forms part of the commensal oral flora of many animals including cats and dogs. It can cause disease in humans and the usual route of infection is through cat or dog bites. *P. multocida* most commonly infects soft tissue, and can also infect the respiratory tract. However, bacteraemia is an uncommon complication.

**Aim**

We aim to look at the epidemiology of *P. multocida* pneumonia with associated bacteraemia in our area, and to describe the clinical features of these cases. This is a retrospective study, reviewing results from Pathology North (Hunter) which provide pathology services for the Hunter New England Local Health District and surrounding areas and across New South Wales (NSW) including Northern NSW, Mid North Coast, Central Coast and Northern Sydney Local Health Districts.

**Methods**

In order to ascertain cases of *P. multocida*, an electronic search was performed on Auslab, our pathology results software. This search identified all episodes across a 16 year period between 30th March 1998 to 5th September 2014. Each patient was counted as a single case regardless of multiple positive samples. Further demographics and clinical details were collected by reviewing handwritten notes during hospital admission and electronic discharge summaries found through our Clinical Applications Portal (CAP).

**Results**

*P. multocida* was isolated in 200 patients from swab, pus, tissue, blood and sputum specimens. Seventeen cases of *P. multocida* bacteraemia occurred, five of which also had pneumonia. This was confirmed in clinical notes and with radiological evidence.

Of particular interest, were two cases with pneumonia that had *P multocida* isolated in both blood cultures and sputum. We describe the diagnosis, therapy, outcome, and pathogenesis of a 28 year old Taiwanese male and a 61 year old Australian female.

**Conclusion**

Our findings show that *P. multocida* pneumonia with associated bacteraemia is uncommon within health services in our area. Our two cases portray an illustration of the varying presentation of this condition.
Prevalence of colonization with Methicillin-resistant Staphylococcus aureus (MRSA) among unselected outpatients in Germany

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In the last years an increasing number of studies have examined the trend of MRSA prevalence in hospitals, but still few attention has been spent to determine the rate in outpatients. Therefore we started an overall MRSA screening on patients aged 18 years and older attending six cooperating general practitioners and one participating radiological group practice in the Rhine-Main area (districts Alzey, Bad Kreuznach, Mainz-Bingen and regional capital Mainz).

Of 1922 persons who were asked to participate 1541 (80.2%) agreed and could be included in the study. They completed a questionnaire in which, among other criteria, risk factors for MRSA according to the KRINKO (national commission for hospital and infection control) recommendation [Epidemiol Bull 2004;46:396] were asked. Further, from 1530 test persons nasal and throat swabs were taken which were cultured separately on Columbia CNA-Agar and selective CHROMagar plates to determine their MRSA status. To control the potential influence of the health personnel on the incidence of MRSA the staff of all practices was voluntarily included in the study as a separate group.

Of the 1530 participants 490 (32.0%) were colonized with S. aureus and 7 (1.4%) of these were resistant to cefoxitin. Thus, the prevalence for MRSA in the entire study population was 0.46%. One case of MRSA transmission between a married couple could be proven by means of spa-typing and PFGE.

Of the 1541 included persons 452 (29.3%) met the criteria of the KRINKO recommendation and therefore would have been screened in case of hospital admission. Of the 7 MRSA positive cases 5 (71.4%) met the KRINKO criteria. In the group of health care workers 50 persons where included of which 18 (36.0%) were colonized with Methicillin-sensitive S. aureus, but no one carried MRSA.

The KRINKO criteria enable us to screen a population cost-effectively, since slightly less than one-third of the study population is needed to be screened to find more than two-thirds of the MRSA colonized persons. Nevertheless one-third of the MRSA cases remains undetected. In comparison to the study of Lietzau et al. [Epidemiol Infect. 2004;132:655-62], our study shows a higher prevalence of MRSA in unselected outpatients (7 MRSA in 490 S. aureus carriers vs. 1 MRSA in 152).
Is it important to report bacterial morphology on desquamated prostate and squamous cell in Semen specimens? A preliminary report.

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Abstract
Background
The Aim of the study is to determine whether bacterial morphology on desquamated prostate and squamous cell could predict the incidence of infection in Semen. According to WHO Guideline, bacterial morphology on desquamated prostate and squamous cell unnecessary to be reported in Sperm analysis. As we know, there's a lot of difficulty to collect semen sample let alone if we collect it for more than one times (for example: semen analysis and semen culture). There for bacterial morphology can be used as an indicator for semen culture.

Material and methods
A cross-sectional study examined bacterial morphology on desquamated prostate and squamous cell of 21 Semens was conducted at Clinical Laboratory. We compared the bacterial morphology at slides (coloured with Giemsa) against semen aerobic bacterial culture results.

Results
There was 10 (47.6%) semen contain bacterial on desquamated prostate and squamous cell and 11 (52.4%) negative. Of the 10 positive slides only 4 (40%) had compatible with culture result which are Staphylococcal infections. The rest of positive slides had negative culture which are contain Streptococcus (4 slides) and Bacillus (2 slides). Of the 11 negative slides only 2 (18.2%) have positive culture result (Streptococcal and Staphylococcal). The Sensitivity, Specificity, PPV and NPV value were 66.67%, 60.00%, 40.00% and 81.82%.

Conclusion
Based on the Positive slides, bacterial morphology on desquamated prostate and squamous cell could be reported as a predictor of infection in Semen. And the Negative slides can rule out incidence of infection.

Key Words: Semen parameters, male genital tract infection, infertility, sperm analysis
Correlation between HBsAg quantitative assay results and HBV genotypes

In chronic HBV

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Globally, HBV infection still a major public health problem and regarding the most important infectious diseases in the era. According to the reports of World Health Organization, a third of the world population has been infected with HBV. Many epidemiological and molecular studies showed that HBV infection has been a significant factor associated with the development of liver cancer (HCC). Accumulated data has been proposed that the important role of genotypes and sub genotypes in clinical outcome and antiviral monitoring of chronic HBV infection. Quantitating and genotyping of HBV DNA levels molecularly is relatively expensive. On the other side, Serum HBsAg quantification has been recently standardized by automated quantitative assays leading to improve interest in the clinical utilization of this marker for diagnosis using sensitive and reliable commercial assays. Several studies have referred to the kinetics of HBsAg to expect a response to antiviral therapy. Thus, a cheaper laboratory test as a surrogate diagnostic marker might simplify our management. Objectives: to evaluate whether quantitative HBsAg levels correlate with HBV genotypes in Malaysian CHB patients.

Patients and Methods: In this cross-sectional study, fifty serum samples from patients with hepatitis B were used, while who were positive for antibodies to HCV and HDV were excluded. The serum HBsAg level was quantified by Elecsysassay. In addition, HBV DNA load was measured by real-time polymerase chain reaction whereas identification of HBV genotypes was done by direct sequencing.

Results: Of 50 patients, 31 were male (44%) and 19 were female (27%); the mean age was 37 ± 12 years. The patients who have HBsAg > 100 IU/ml were (42). Eighty percent was HBeAg-negative (40) while 20% (10) was positive. Genotyping results showed that 22 patients have genotype C with percentage (44%), and 18 patients with genotype B (36%). By Mann-Whitney test, HBsAg titre was correlated significantly between HBV genotype C & B. (P < 0.05). By Non parameter T test, there was no significant correlation between HBsAg level and HBV DNA levels (P<0.05). Conclusions: the significant observed association between levels of HBsAg and HBV genotypes was found. Hence, HBsAg level could be a useful indicator to predict the genotype B and C of Hepatitis B virus and ultimately predict how antiviral drugs success in treatment.
HIV prevalence over the recent decade: The Asia Pacific perspective
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Introduction: In excess of 35 million people worldwide are currently living with HIV/AIDS. Despite HIV prevalence exhibiting downward trends in most countries, millions of infected individuals are still not gaining access to life-saving anti-retroviral therapy resulting in countless premature deaths. In the Asia Pacific region alone, close to 5 million people live with HIV with over 90% of those affected residing in India, China, Indonesia, Vietnam, Malaysia, Myanmar and Thailand.

Objective: This analysis was performed to assess trends in HIV prevalence of people aged 15-49 years across countries in the Asia Pacific region.

Methods: HIV prevalence data of people aged 15-49 years pertaining to particular years (2001, 2007 and 2011) were obtained from the databases of selected countries and the World Health Organization (WHO)/UNAIDS. The countries chosen for comparative HIV prevalence analysis included Thailand, Papua New Guinea (PNG), Cambodia, Myanmar, Vietnam, Malaysia, Indonesia, India, Japan, Philippines, New Zealand, China, Republic of Korea and Australia. HIV prevalence pertaining to people aged 15-49 years in individual countries at specified time-points was analysed utilising two-way ANOVA and subsequently corrected for multiple comparisons. A critical p<0.05 was considered statistically significant with selected trends in HIV prevalence described.

Results: Following multiple comparison analysis, Cambodia exhibited significant declines in HIV prevalence of people aged 15-49 years over two time-periods: from 2001 to 2007 (2,774 per 100,000 population vs. 800 per 100,000 population; p<0.01), and 2001 to 2011 (2,774 per 100,000 population vs. 600 per 100,000 population; p<0.01), respectively. Hence, Cambodia experienced a 71% decline in HIV prevalence from 2001 to 2007 in the prescribed population; representing the greatest decline of all countries analysed. Additionally, Myanmar exhibited a 70% decrease in HIV prevalence of people aged 15-49 years from 2001 to 2011 (1,979 per 100,000 population vs. 600 per 100,000 population; p<0.05). PNG showed a 134% increase in HIV prevalence from 2001 to 2007, and a subsequent decrease almost returning to baseline in 2011. However, the changes in HIV prevalence exhibited over both corresponding time periods failed to reach significance (p>0.05).

Conclusion: HIV prevalence in the Asia Pacific region in certainly exhibiting a significant downward trend especially among the heavily burdened countries. However, the total number of HIV cases in the region has yet to peak. Thus, it warrants that complacency does not ensue and that every effort is undertaken ensuring the continued decline in HIV prevalence by persisting and expanding on population-wide health education initiatives.

Reference (8)
Staphylococcus aureus Carriage In Children

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Abstract

Background- Staphylococcus aureus is a common cause of disease, particularly in colonized persons. Nasal colonization of S. aureus is common in children, thus children is the important reservoir of S. aureus for future infections in the community. Based on reports from other countries, the rate of S. aureus nasal colonization in healthy children were from 18% to 30%. One to three percent of them were colonized by Methicillin-resistant Staphylococcus aureus (MRSA) (Lamaro-Cardoso et al. 2009, Miller et al. 2011). Overcrowding and natural intimacy of children may facilitate transmission of infections to others. Although MRSA infection has become increasingly reported, population-based S. aureus and MRSA colonization estimates are lacking. This main objective of this study were to determine the prevalence of S. aureus carriage among children.

Method- Nasal samples for S. aureus culture were obtained from 250 children (aged 4 to 6 years old) from 3 kindergartens in the Klang Valley, after consented by their parents. Swabs were transported in Stuart medium, and inoculated on mannitol salt agar within 3 hours. Identification and disk diffusion test were done according to guidelines.

Results- The prevalence of S. aureus carriage in kindergarten A, B and C were 15% (18/120), 16.9% (12/71) and 28.8% (17/59) respectively. Overall prevalence was 18.8% (47/250). No MRSA was isolated from A and B. 23.5% (4/17) of S.aureus from C were resistant to cefoxitin (MRSA). All MRSA were sensitive to vancomycin.

Conclusions- Prevalence of S. aureus nasal colonization in healthy children in the Klang Valley is similar to worldwide prevalence. As colonization typically precedes infection, hand hygiene education is important to limit contact transmission.
HHV-8 AMONG OMANI HIV, RENAL TRANSPLANT PATIENTS AND HEALTHY INDIVIDUALS

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Many diseases are thought to be associated with Human Herpes virus type 8 or Kaposi Sarcoma associated Herpes Virus (HHV-8/KSHV). Kaposi sarcomas (KS), Primary Effusion Lymphoma (PEL) and Multicentric Castelman's Disease are the main clinical presentations that are associated with HHV-8 infection. They usually affect immunosuppressed patients such as HIV patients and organ transplant recipients.

Three different Omani groups were chosen to investigate the occurrence of HHV-8 infection: HIV infected individuals, renal transplant patients and healthy individuals. Factors that might contribute to HHV-8 infection were also studied.

A total of 302 paired serum and Peripheral Blood Mononuclear Cells (PBMCs) samples were collected from hospitals and clinics. Serum samples were tested using KSHV ELISA Kits that utilizes the whole virus lysate to investigate the presence of HHV-8 antibodies. HHV-8 viral loads were measured from 302 PBMCs samples using Real Time PCR. Moreover, HHV-8 viral loads were measured from 17 serum samples and 11 whole blood specimens that belonged to HHV-8 seropositive individuals using Real Time PCR.

The overall HHV-8 seroprevalence was found to be 5.6% in the 302 tested serum samples. HHV-8 was highly prevalent among HIV patients (16%) and Renal Transplant Patients (6%) compared to healthy group (0.71%) with an overall p-value = 0.003. No significant differences were found between different demographic factors.

Additionally, no significant differences were found between clinical data for HIV or Renal transplant patients such as CD4 counts, HIV viral loads, and type of antiretroviral therapy, frequency of hemodialysis, date and place of transplantation, type of donors, HBV, HCV and type of immunosuppressive therapy.

The occurrence of HHV-8 DNA isolated from PBMCs was 3.2% (2/62) among HIV patients, 1% (1/100) among renal transplant patients compared to zero % (0/140) in the control group. Only one serum sample was shown to be positive to HHV-8 DNA that belonged to a renal transplant patient who previously was diagnosed with Kaposi Sarcoma and none of the whole blood samples showed positivity to HHV-8 DNA.

According to the results obtained, KSHV/HHV-8 appears to be rare among the Omani population. Therefore, Oman could be classified as a low prevalence area for HHV-8.

Serological and molecular screening of donors might be of a critical value especially in endemic areas of HHV-8. Screening of HIV patients could play a role in identifying the patients who are at risk of developing KS and helpful in monitoring those patients clinically for a better patient management.
IMPACT OF PUTATIVE BACTEROCINS AGAINST MULTIDRUG RESISTANT CLINICAL ISOLATES

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ABSTRACT

Background: Bacteriocins are well known for their antibacterial activity against multidrug resistant strains (MDRs). Lactobacilli are known friendly bacteria for their antibacterial activities against pathogens. The antibacterial activity of different strains of Lactobacilli was analyzed against multidrug resistant strains (MDRs).

Objectives: Screening and characterization of multidrug resistant strains (MDRs). Characterization of the putative bacteriocins produced by selected bacteria (Lactobacilli). Finally check the antibacterial effect of putative bacteriocins against multidrug resistant strains (MDRs).
**Materials and Methods:** Multidrug resistant clinical isolates were selected on the basis of their MAR index. Well-Diffusion assay was used for screening of putative bacteriocins produced by *Lactobacillus* strains against MDRs.

**Results:** Multidrug resistant strains were selected based on MAR (Multiple antibiotic resistance) index. Five bacteriocins obtained from *Lactobacillus* strains isolated from commercial products. These bacteriocins showed a strong anti-bacterial activity against selected MDRs. Decrease in zone sizes was observed when putative bacteriocins were treated with heat, SDS and Protinase k. It was observed that *Lactobacillus* showed a significant antibacterial activity in-vitro in the presence of putative bacteriocins against selected MDRs and further experiments are under process.

**Conclusion:** Putative bacteriocins produced by *Lactobacilli* exhibit significant antibacterial activity against MDRs. The peptidal component of these bacteriocins can be used as an alternative therapy. Hence, it is necessary to purify the antibacterial molecule out of putative bacteriocin for further analysis.

**Key Words:** MDRs, Bacteriocins, *Lactobacilli*, Antibiotic Resistance, MAR, Antibacterial Activity.
In-house ELISA screening using a locally-isolated leptospiral antigen in Malaysia: Determination of its cut-off points

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Leptospirosis is a zoonotic disease of worldwide distribution which is caused by Leptospira. Diagnosis of leptospirosis depends mainly on detection of antibody because this organism is very difficult to isolate. Microscopic agglutination test (MAT) is the gold standard of serological diagnostic test of leptospirosis. However it is time-consuming, labour-intensive and not useful for acute patient management. Rapid diagnosis of leptospirosis is important for early treatment which can significantly reduce morbidity and mortality. In this study, the sensitivity, specificity and cut-off point were determined for an in-house immunoglobulin M enzyme-linked immunosorbent assay (IgM ELISA) using a locally isolated leptospiral strain IMR/175 as the antigen for the detection of IgM in suspected cases of leptospirosis. Single serum samples from 270 suspected leptospirosis patients were subjected to MAT and the in-house ELISA. The cut-off values, sensitivity and specificity were determined by Receiver Operating Characteristic curves analysis. The diagnostic performance of the IgM ELISA assay as indicated by the area under the curve was 0.953 (95% Confidence Interval: 0.928, 0.978). The optimal cut-off value of the IgM ELISA test was identified by using the point with the highest Youden Index. The positive and negative cut-off points were set at of 0.55 (sensitivity: 90.4%, specificity: 87.7%) and 0.45 (sensitivity: 96.2%, specificity: 58.8%) respectively. In conclusion, using these cut-off points this newly developed IgM ELISA assay can be a valuable screening test for leptospirosis.
Immunization with *Staphylococcus aureus* ghost vaccine protected rats from virulent challenge


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*Staphylococcus aureus*, a Gram-positive bacterium, is one of the important bacterial pathogens which causes a wide range of infections in human and animals. Bacterial ghosts (BGs) are empty bacterial cell envelopes and well-represented as a novel vaccine candidates. In this study, we examined the immunogenicity and protective efficacy of *S. aureus* ghosts (SAGs) against virulent challenge in rats. The non-living SAGs were generated by using the MIC of sodium hydroxide (NaOH). The transmembrane lysis tunnel structure in SAGs was visualized by scanning electron microscopy (SEM). To investigate the SAGs as a vaccine candidate, rats were divided into four groups: group A (non-immunized control), group B (orally immunized), group C (subcutaneously immunized) and group D (intravenously immunized). The specific IgG antibody response of SAG vaccine was significantly increased in immunized groups as compared to non-immunized control group (*P* < 0.05). Moreover, significant increase in population of CD4⁺ and CD8⁺ T-cells were observed in all three immunized groups (*P* < 0.05). We also found that the serum bactericidal antibodies were significantly elicited in immunized groups compared to the non-immunized control group (*P* < 0.05). Most importantly, bacterial loads in immunized groups were significantly lower than non-immunized control group (*P* < 0.01). These results suggest that immunization with SAGs induces immune response and provides protection against virulent *S. aureus* challenge.

*Young Investigator Award (I wish to be considered for the Young Investigator Award.*)
Recalcitrant coagulase-negative methicillin sensitive *Staphylococcus aureus* in an extremely low-birth-weight pre-term infant at University Malaya Medical Center

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**Background:** Infections caused by *Staphylococcus aureus* often involve complex treatment and control. Virulent *S. aureus* is commonly positive for coagulase test, whilst non-virulent isolates are coagulase negative. Reports on coagulase-negative variants of *S. aureus* and their role in clinical disease in neonates is very scanty.

**Case presentation:** Here we describe a case of an extremely low-birth-weight pre-term infant who had persistent recalcitrant infection with coagulase-negative methicillin-sensitive *S. aureus*, that caused clinical sepsis, thrombocytopenia and persistence in blood cultures for 7 weeks. The API STAPH-Ident system (Bio-Merieux) and 16S rRNA gene sequencing were used to confirm identity. Parallel use of blood culture yielded an isolate with the same phenotypic identity. **Conclusion:** Past reports have shown the recovery of some uncommon strains of *S.aureus*, which required longer incubation periods to yield a positive coagulase reaction. Therefore, prompt identification of uncommon phenotypes of *S. aureus* needs the use of appropriate molecular diagnostics to necessitate timely treatment of life-threatening systemic infections especially in neonates.

Keywords: 16S rRNA gene sequencing; coagulase negative; neonatal infections; sepsis.