Purple urine bag syndrome, a rare urological phenomenon: Case report from a nursing home in Malaysia

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INTRODUCTION

Purple urine bag syndrome (PUBS), as the name implies produces purplish discoloration of the urine. It is usually benign in nature, but the appearance of the urine may be alarming to the patient, family and health-care workers. From the literature research, there were no publications on PUBS in Malaysia; however we believe that it is underreported. We present a unique case of this rare condition occurring in 68 year old man, a nursing home resident.

CASE REPORT

A 67-year-old man has been bed bound for 2 years following motor vehicular accident and on suprapubic catheter. He presented with purplish discoloration of the urine (Fig 1) for the last 10 days with no evidence of sepsis. The suprapubic catheter was last changed a month ago. Laboratory investigations revealed hemoglobin of 10.9 g/dL, total white cell count was 8.1 x 10⁹/liter, platelet of 342 x 10⁹/liter, blood urea of 4.5 mmol/L and creatinine of 88umol/L. Urine analysis showed urine pH of 9, leucocyte was 3+, erythrocyte was 4+, and nitrite was negative.

Patient was started on intravenous ceftriaxone 2 gram daily to cover for possible urinary tract infection while waiting for cultures. Following hydration and replacement of suprapubic catheter, the urine became clear the next day. Patient remained afebrile in the ward and urine persistently clear thereafter. The urine culture showed mixed growth of 10,000-100,000/ml urine while the blood culture had no growth. He was discharged well with 5 days of oral cefuroxime 500mg BD.

CONCLUSION

PUBS, is a benign condition. In Malaysia, there is growing number of nursing homes and awareness needs to be addressed to health-care workers and on PUBS prevention. Reducing the duration of catheterization, improving urological sanitation and control of constipation by appropriate nutritional management are key elements for the prevention of PUBS.
Surface interactions between periodontal pathogens *Porphyromonas gingivalis* and *Tannerella forsythia*

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**Objective:** Preliminary data have implicated surface interactions between two crucial periodontal pathogens, *Porphyromonas gingivalis* (*Pg*) and *Tannerella forsythia* (*Tf*). The purpose of this study was to identify surface proteins from *Pg* and *Tf* that are involved in this physical interaction.

**Method:** Coaggregation activity between *Pg* and *Tf* were observed by negative staining. After *Tf* and *Pg* surface proteins were labeled with Sulfo-NHS-LC-biotin, these were incubated with whole *Pg* or *Tf* cells, respectively. The resultant *Pg* or *Tf* cells to which biotinylated *Tf* or *Pg* proteins are attached were separated by SDS-PAGE and transferred to PVDF membrane. Subsequently, biotinylated surface proteins from *Tf* and *Pg* were detected with streptavidin-HRP and 4-chloro-1-naphthol.

**Result:** Inter-bacterial binding between *Pg* and *Tf* was confirmed by negative staining electron microscopy. *Tf* surface proteins bound to *Pg* cells were detected using 1-D and 2-D gel electrophoresis, as three major surface proteins with molecular masses of 160, 100, and 60 kDa that are found to bind to *Pg* cells. In 2-D gel electrophoresis, proteins >100 kDa were detected. Also, *Pg* surface proteins ranging from 40 to 120 kDa were found to bind to *Tf* cells.

**Conclusion:** The surface proteins of *Pg* and *Tf* cells were biotinylated and successful biotinylation was confirmed by a confocal microscopy. Major surface *Pg* and *Tf* proteins were identified, which are involved in physical interaction with *Tf* and *Pg*, respectively, leading to coaggregation. Further characterization of major surface proteins would help define specific roles of these proteins in coaggregation between *Pg* and *Tf*.
Abstract

**THE CORRELATION BETWEEN POLYMORPHISM OF PROMOTOR-308G/A GENE TUMOR NECROSIS FACTOR-α WITH TNF-α SERUM LEVEL ON SEPSIS SEVERITY AT DR. MOHAMMAD HOESIN HOSPITAL PALEMBANG INDONESIA**

Harun Hudari, Susi Kurnia, Rizky Perdana, Akmal Sya’roni

**Objective:** To define the connection between promoter-308 g/a gen TNF-α polymorphism with TNF-α serum level on sepsis severity at dr. Mohammad Hoesin hospital, Palembang

**Method:** This is analytic observational study with case control Study. The sample was all patient with severe sepsis according to ACCP/SCCM consensus at ward and ICU, sample from malay Indonesia race. The exclusion criteria was patient under 16 years and over 60 years old, patient with immunosupresion or immunocompromise. The sample was analized using ELISA to gather TNF-α serum level. Polymorphism of TNF-α gene was identified using dengan RFLP technique.

**Result:** Fourty subject were obtained and divide into 2 grups, sepsis and severe sepsis. Twenty patient (58,8%) with TNF-α serum rising has genotip AA, the other 14 (41,2) has genotip GG. On patient with rising level of TNF-α, G alel obtained at 33,3% and alel A at 66,7%. there are no correlation between TNF-α 308 with TNF-α serum (p=0,609), odds ratio 1,4 (0,384-5,105; 95% CI). there are significance correlation between polymorphism gene TNF-α 307 with severity of sepsis for genotip point of view (p=0,000) with odds ratio 57,0 (6.000-541.466; 95% CI) and also from alel point of view <p=0,000) with odds ratio 57,0 (11.602-280.034; 95% CI). Eighteen subject (90%) on sepsis group has no inclination of TNF-α level and 16 ( 80%) from severe sepsis group. there are no significance connection between TNF-α level with the proportion of severity sepsis (p=0,331), odds ratio 2,250 (0,362-13,971; 95% CI. patient has bigger risk as much as 2250 times on become sepsis at patient high rising level of TNF-α.

**Conclusion:** Polymorphism of promoter-308 g/a gen TNF-α G/A has no correlation with serum level TNF-α, but has positif correlation with severity of sepsis

**Keyword:** Polymorphism promotor gene TNF-α G/A, TNF-α serum level, sepsis severity.
Long-term Stability and In-vitro fungal activity of Voriconazole eye drop solution (1%)
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Background: Fungal keratitis is recognized worldwide as a vision threatening corneal infections. The combined systemic and topical administration of voriconazole has successfully been used to treat fungal keratitis. Due to lack of commercial source of voriconazole eye drops, it has been prepared as an eye drop solution (1%) from the systemic injection powder. There are negligible data in the literature related to the long-term stability of prepared solution and its clinical use. Hence, aim of this study was to check the stability and in-vitro fungal activity of voriconazole powder for eye drops 1% w/v by both chemical and microbiological methods. Methods: Stability, pH, and clarity were analyzed for reconstituted voriconazole eye drops 1% under room temperatures (28 – 30˚C) and refrigerated conditions (2 – 8˚C). The in-vitro efficacy of voriconazole eye drops analyzed using the standard national committee for clinical laboratory standards method against most common fungal corneal isolates. All the tests performed every 7 days up to 30 days. Results: Voriconazole concentrations found within the specifications (85 – 110%) in both conditions, pH of the solutions within the acceptance limit (pH 5 – 6.5) in both conditions. The antifungal activities of reconstituted voriconazole remained stable during all four weeks, the range of MIC values are same for both conditions. Conclusion: The reconstituted voriconazole 1% eye drops preparation remained stable, with full antifungal activity for 28 days when stored both at room temperature and refrigeration conditions. Data strongly suggest that prepared voriconazole eye drops can be use it for topical application up to 28 days in room temperature or refrigerated conditions.
Correlating Echinocandin MIC and Candida glabrata fks1 Mutations.
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Objectives
To study the correlation between echinocandin MIC and fks mutation for Candida glabrata.

Methods
Spontaneous echinocandin-resistant mutants of C. glabrata strains ATCC90030 were isolated by plating 10 μl (~10^9 cells) of an overnight liquid Sabouraudbroth culture onto Sabourauddextrose agar plates containing 0.06, 0.12, 0.25, 0.5, 1 or 2 μg/ml micafungin or anidulafungin. Serial dilutions of the overnight cultures were plated onto the Sabourauddextrose agar plates without antibiotic selection to determine the starting cell counts. Selection plates were incubated for 7 days at 30°C. From the selection plates, selected colonies were re-inoculated on fresh Sabourauddextrose agar plates containing 0.25 μg/ml micafungin or 0.25 μg/ml anidulafungin to confirm the resistant phenotype. The echinocandin MIC of the resistant isolates were studied. PCR and sequencing were used to detect mutations in the hotspot regions of the two fks genes, fks1 and fks2.

Results
Seventeen spontaneous echinocandin-resistant mutants of C. glabrata strains ATCC90030, namely CGMT1 to CGMT17, were isolated (See Table 1). Five different fks1 mutations were detected. No mutation was detected in fks2 hotspot regions. The fks1 mutant isolates showed 2- to 30-fold MIC increases for anidulafungin and micafungin, relative to the C. glabrata strains ATCC90030. The most significant MIC increases was related to the deletion mutation of FKS1 at Phe625 (32-fold for micafungin and 16-fold for anidulafungin), whereas the other mutations in FKS1 (F625Y, S629F, S629P, P633T) accounted for smaller increases (2- to 4-fold for both echinocandins).
Table 1 *In vitro* susceptibilities of the studied isolates to micafungin and anidulafungin, and the FKS hotspot alterations in the isolates

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC results(μg/ml)*</th>
<th>FKS1 HSb</th>
<th>FKS1 HSb</th>
<th>FKS2 HSb</th>
<th>FKS2 HSb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micafungin</td>
<td>Anidulafungin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 90030</td>
<td>0.06 (S)</td>
<td>0.06 (S)</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT1</td>
<td>2 (R)</td>
<td>1 (R)</td>
<td>F625Δ</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT2</td>
<td>2 (R)</td>
<td>1 (R)</td>
<td>F625Δ</td>
<td>WT</td>
<td>WT</td>
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<tr>
<td>CGMT3</td>
<td>2 (R)</td>
<td>1 (R)</td>
<td>F625Δ</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT4</td>
<td>2 (R)</td>
<td>1 (R)</td>
<td>F625Δ</td>
<td>WT</td>
<td>WT</td>
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<tr>
<td>CGMT5</td>
<td>2 (R)</td>
<td>1 (R)</td>
<td>F625Δ</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT6</td>
<td>2 (R)</td>
<td>1 (R)</td>
<td>F625Δ</td>
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</tr>
<tr>
<td>CGMT7</td>
<td>2 (R)</td>
<td>1 (R)</td>
<td>F625Δ</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT8</td>
<td>2 (R)</td>
<td>1 (R)</td>
<td>F625Δ</td>
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<td>CGMT9</td>
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<td>1 (R)</td>
<td>F625Δ</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT10</td>
<td>2 (R)</td>
<td>1 (R)</td>
<td>F625Δ</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT12</td>
<td>0.12 (I)</td>
<td>0.25 (I)</td>
<td>S629P</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT13</td>
<td><strong>0.25 (R)</strong></td>
<td>0.25 (I)</td>
<td>F625Y</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT14</td>
<td>0.12 (I)</td>
<td>0.25 (I)</td>
<td>S629P</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT15</td>
<td>0.12 (I)</td>
<td>0.12 (S)</td>
<td>S629F</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT16</td>
<td>0.12 (I)</td>
<td>0.25 (I)</td>
<td>S629P</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>CGMT17</td>
<td>0.12 (I)</td>
<td>0.12 (S)</td>
<td>P633T</td>
<td>WT</td>
<td>WT</td>
</tr>
</tbody>
</table>

*a*studied by CLSI M27-A3 method (microdilution) with interpretation criteria CLSI M27-S4; R: resistant; I: intermediate; S: sensitive.

*b*HS1, Hotspot1; HS2, Hotspot 2; WT, wild-type; Δ, deletion.

**Conclusion**

The echinocandin resistance was demonstrated to be correlated to mutations in the *fks1* of *C. glabrata* and the MICs of echinocandins varied with the specific *fks1* mutations.
Lock, Stock and Dual Smoking Antibiotics: Successful Novel CVC Line Salvage using combination Antibiotic Lock Therapy (ALT) in Haemodialysis

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Background and rationale
Catheter-related bacteraemia (CRB), associated with a microbial biofilm, is a serious complication in central venous catheter (CVC) use in haemodialysis patients. Despite significant morbidity, mortality and economic burden, research into use of ALT in this setting remains unsatisfactory. We describe two examples of ALT both using dual antibiotics for catheter salvage. One particular ALT’s originality prompted its selection for poster presentation at Antimicrobials 2014.

Methods
Two haemodialysis patients, both on three times a week dialysis, were receiving a gentamicin (GEN) lock as per unit protocol. However, these patients developed CRB and the decision was made to salvage the CVC’s in both cases.

Case 1: This patient developed *Citrobacter freundii* CRB and infectious disease (ID) recommended amikacin (AMK) as ALT salvage therapy. A few weeks later, he developed another CRB due to *Corynebacterium jeikeium* sensitive to vancomycin (VAN) and dual (AMK+VAN) ALT was suggested. Although evidence supported compatibility of the antibiotics in lock solution, there were no studies to confirm the combination’s efficacy in ALT. Since microbial antibiotic susceptibility confirmed this as a logical ALT therapy, it was administered. The CVC remained successfully in situ for another couple of weeks, but the decision to remove the CVC was eventually made in the light of suitable native arteriovenous access.

Case 2: This patient had developed *Achromobacter xylosoxidans* CRB which already proved resistant to GEN but susceptible to piperacillin-tazobactam (TAZ). Asper unit protocol TAZ ALT was commenced. Three months later the *Achromobacter xylosoxidans* CRB returned, with again, susceptibility to TAZ, but also susceptible to trimethoprim-sulfamethoxazole (TMP-SMX). Poor penetration of the biofilm by TAZ was suspected. Removal of the catheter was suggested by ID, however a switch to an innovative TMP-SMX antibiotic lock and concurrent oral TMP-SMX therapy was instituted. Ongoing treatment with this novel regime resulted in successful CRB treatment and salvage of the CVC.

Implications
The development of these two ALT’s for haemodialysis patients may have potential for application in other health care scenarios (palliative and total parenteral nutrition).

Key Message
This case highlights the need for more research into the antibiotic lock options for contaminated CVC’s.
Prevalence of CMV antibodies among women of childbearing age in referral Shahid Mofateh Clinic Yasuj Iran
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BACKGROUND:
Cytomegalovirus (CMV) is the most frequent cause of congenital infection. The objective of this study was to evaluate seroprevalence of CMV infection among women of childbearing age in Yasuj, Iran.

METHODS:
This cross-sectional study was conducted on 94 systematic randomly selected women in Sh.Mofateh Clinic Yasuj, Iran between December 2012 and January 2014, sera from women were tested by enzyme immunoassay for CMV IgG and IgM. Variables collected were age, geographic origin, lifestyle, work characteristics, socioeconomic status, gravidity, parity and number of children at home.

RESULTS:
93 patients (98.9%) of the participants in this study were positive for anti-CMV IgG antibody, whereas the IgM antibody in only one patient (1.1 %) was positive and others were negative. Also, the titer of IgG and IgM antibodies and clinical manifestations associated with demographic variables was not significant (p>0.05).

CONCLUSION:
women of childbearing age in Yasuj have one of the highest reported CMV sero-prevalence rates worldwide, indicating high circulation of CMV within the community. So these women are susceptible to infection during pregnancy and congenital anomaly of their neonates.

Key words: Women, cytomegalovirus, Seroprevalence
Device related infections at Duzce university hospital of intensive care units: a 6-year experience

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Background: Device Related Infections (DRIs), such as catheter-associated urinary tract infections (CAUTIs), central line-associated bloodstream infections (CABSIs), and ventilator-associated pneumonia (VAP) pose the greatest threat to patient safety in intensive care units (ICUs). The purpose of this study was to evaluate DRIs according to years in ICUs of Duzce University Hospital, in Turkey.

Methods: A prospective surveillance was performed to determine DRIs rates during 2007-2012. Hospital infections were identified according to the definitions of Centers for Disease Control and Prevention. The incidence densities of DRIs were calculated as the number of infections per 1000 device-days.

Results: During the six years period, 2921 patients were followed in ICUs and 1045 hospital infection episodes were detected in 31311 patient-days. The overall hospital infections incidence density was 33.4 for ICUs. Of these infections, 772 were identified as DRIs. Of 772 DRIs, 526 were VAP, 159 were CAUTIs and 87 were CABSIs. The incidence density and device utilization ratio were 25 (0.66) for VAP, 7 (0.37) for CABSIs and 5 (0.98) for CAUTIs in six years (Table 1). There were no statistically significant differences in annual incidence densities of DRIs over time from the beginning of the study to the end of the study. (VAP: p=0.118, CABSIs: p=0.244, CAUTIs: p=0.932).

Table 1: The incidence density and device utilization ratio of DRIs for six years.

<table>
<thead>
<tr>
<th>DRIs</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAP</td>
<td>20(0.58)</td>
<td>16(0.74)</td>
<td>19(0.71)</td>
<td>30(0.70)</td>
<td>28(0.65)</td>
<td>31(0.56)</td>
<td>25(0.66)</td>
</tr>
<tr>
<td>CABSIs</td>
<td>15(0.29)</td>
<td>8(0.40)</td>
<td>6(0.41)</td>
<td>7(0.39)</td>
<td>7(0.40)</td>
<td>7(0.32)</td>
<td>7(0.37)</td>
</tr>
<tr>
<td>CAUTIs</td>
<td>40(0.96)</td>
<td>6(0.99)</td>
<td>5(0.96)</td>
<td>5(0.99)</td>
<td>4(0.99)</td>
<td>7(0.99)</td>
<td>5(0.98)</td>
</tr>
</tbody>
</table>

Conclusion: VAP was among the most common DRIs in ICUs. Accurate determination of the invasive procedure indications, removal of the device as early as possible will decrease the rate of DRIs. To reduce infection rates and improve quality of care in ICUs in developing countries, comprehensive infection control programs are essential.

Key words: Hospital infection, Nosocomial infection, Healthcare-acquired infection, Device related infection
Utility of PCR targeting IS6110 and MPT64 genes in the diagnosis of extrapulmonary tuberculosis
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Background: Tuberculosis remains a global health problem. An estimated 8.7 million new cases of TB and 1.4 million deaths occurred in 2011. Burden being greatest in Asia and Africa, India and China together account for 40% of the world’s cases. Definitive diagnosis of TB involves demonstration of Mycobacterium tuberculosis by microbiological or histopathological methods. Clinical presentation of extra pulmonary tuberculosis (EPTB) being atypical especially when the disease involves obscure occult sites; clinicians more often rely upon non-conventional diagnostic methods. The aim of this study was to evaluate the diagnostic performance of polymerase chain reaction (PCR) targeting IS6110 and MPT64 genes in EPTB.

Methods: Clinical specimens from 150 patients with presumptive clinical diagnosis of EPTB were prospectively examined by Ziehl-Neelsen staining and mycobacterial culture on Lowenstein Jensen medium. Cytological and histopathological examinations were done in cases of lymph node aspirates and endometrial biopsies respectively. DNA from all the specimens was amplified by conventional PCR using primers targeting 123 bp and 240 bp fragments of IS6110 and MPT64 genes respectively. Real time PCR targeting MPT64 gene was also performed. The criteria for a positive diagnosis of TB were positive culture with or without positive acid fast staining and suggestive histopathology; or compatible clinical and radio imaging evidence with clinical improvement on trial of anti-tuberculosis treatment.

Results: Lymph node aspirates showed highest AFB positivity (33.3%) by Z-N staining being positive in 30 out of 150 (20%) samples whereas ascitic fluid samples showed highest positivity (33.3%) by culture which was seen in 27 out of 150 (18%) samples. 24 out of 60 (40%) lymph node aspirates were positive by histopathology as compared to 6 out of 45 (13.3%) endometrial biopsies. The combined sensitivity of the three conventional diagnostic methods used in the study was 64.3%. 76 (50.7%) samples showed positive results with PCR targeting IS6110 gene with CSF (71.4%) and synovial fluid samples (75%) showing highest positivity. 74 (49.3%) samples showed positive results with PCR targeting MPT64 gene with lymph node aspirates (68.3%) and synovial fluid samples (75%) showing highest positivity. PCR for IS6110 gene was more sensitive (82.1%) as compared to PCR for MPT64 gene (81%). Real time PCR for MPT64 gene was found to be most sensitive (87%) with the overall sensitivity of all PCRs being 89.3%.

Conclusion: Amplification techniques have attracted considerable interest for diagnosis of TB, though more studies are required to establish their role in replacing traditional methods of diagnosis.
Combination of Fosfomycin and Levofloxacin for Multidrug-Resistant *Pseudomonas aeruginosa* Sepsis

A Case Report

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**Background.** Sepsis due to multidrug-resistant (MDR) *Pseudomonas aeruginosa* is associated with high mortality rates and considerable therapeutic challenge because no new antipseudomonal agents are expected to be available in the near future. Herein we present a case report of sepsis caused by MDR *P. aeruginosa* which was successfully treated empirically with combination of fosfomycin and levofloxacin.

**Case Illustration.** A 36-year-old man was referred to *Indonesia Central Army Hospital Gatot Soebroto* from Papua, with diagnosis of severe sepsis and hospital acquired pneumonia after suffering from severe malaria falciparum. He had been treated for one month in an intensive care unit (ICU) with levofloxacin for twenty three days and combination of levofloxacin and meropenem for the last eight days. His sputum culture (taken in our hospital) showed two isolates. First was *Pseudomonas spp* which was sensitive only to fosfomycin, cefpirome, and cefoperazone. Second isolate was *Candida albicans* which was sensitive to amphotericin B and imidazole class. Urine culture showed *P. aeruginosa* which was sensitive to amikacin, fosfomycin, cefpirome, and cefoperazone. The antimicrobial regimen was changed to cefoperazone-sulbactam 1 gram thrice daily, amikacin 1 gram once daily, and intravenous fluconazole 400 mg once daily. On day 9 of ICU therapy, his tracheostomy swab culture detected *Pseudomonas aeruginosa* which was resistant to all class of antibiotics and intermediate sensitive to cefoperazone. His procalcitonin level increased from 2 to 10 ng/mL and chest x-ray showed new pleural effusion on the left lung. The antibiotic regimen was changed to fosfomycin 2 gram twice daily and levofloxacin 750 mg once daily for seven days. Clinical cure was achieved and his antibiotic regimen was switched to oral cefditoren.
Discussion
Fosfomycin, an old class of antibiotic, has been shown in several studies to have a synergistic effect when combined with other antibiotics against *P. aeruginosa*. Combination of fosfomycin and levofloxacin has been shown to be effective in eradicating sessile cells of *P. aeruginosa* in biofilm-associated infectious diseases. The effectiveness of fosfomycin combined with levofloxacin against MDR *P. aeruginosa* isolates, using the efficacy time index assay, was 66.7%.

Conclusion
Combination of fosfomycin and levofloxacin could be an alternative option for treating sepsis with MDR *P. aeruginosa*.

Keywords: Sepsis, multidrug-resistant, *Pseudomonas aeruginosa*, fosfomycin, levofloxacin
Detection of *Trichomonas vaginalis* in benign hyperplastic prostate tissue

**Abstract**

Prostate cancer and benign prostatic hyperplasia (BPH) represents the most common urologic disease among the elderly males resulting in more than 2 million visits per year. BPH affects about one-quarter of men in their 50s. The pathogenesis of BPH is not yet completely understood however, the role of chronic inflammation is emerging as an important factor in BPH development and progression. Recently, the studies have found that *T. vaginalis* may be associated with asymptomatic infections in 50-75% of infected men. In this study we investigated the possibility of asymptomatic persistence of *T. vaginalis* in the prostate gland using benign hyperplastic prostate tissue as prostate condition other than clinical prostatitis.

**Study subjects & Methods:** We investigated the occurrence of *T. vaginalis* in prostate tissue of 75 men of >50 years of age suspected and treated for BPH by transurethral resection of the prostate at the Mubarak Al-Kabir Teaching Hospital, Kuwait. The presence of *T. vaginalis* infection in the prostate tissue was determined by PCR analysis of the DNA extracted from the tissue and Immunocytochemistry of the tissue sections of the prostate tissue. In addition, P16 antigen was also detected in the tissue sections. The antibodies to *T. vaginalis* were also determined in blood.

**Results:** We detected *T. vaginalis* DNA in 18 of 75 (24%) and P16 antigen in 16/75 (21%) of BPH tissue samples, of which only 7 (39%) BPH tissues were positive by immunocytochemistry. In addition, three *T. vaginalis* DNA-negative prostate tissues were also positive immunocytochemistry. *T. vaginalis*-specific antibodies with predominantly IgG4 antibodies were detected in 23 (31%) cases.

**Conclusion:** Our preliminary study suggests a direct evidence of *T. vaginalis* in BPH tissues with no clinical signs of prostatitis. We hypothesize that chronic *T. vaginalis* infection of prostate tissue may lead to BPH in elderly people.
ABSTRACT

THE EFFECTIVENESS OF HANDRUB PRACTICE IN PREVENTING INFLUENZA LIKE ILLNESS AMONG MALAYSIAN HAJJ PILGRIMS 2013

Zeti Norfidiyati Ayub, Suhana Hashim, Habsah Hasan, Aniza Abdul Aziz, Syed Hatim@ Nyi Nyi Naing, Zeehaidah Mohamed, Azian Harun, Zaidah Abdul Rahman, Nabilah Ismail, Siti Suraiya Md Noor.

INTRODUCTION

Influenza-like illness (ILI) was defined as triad of cough, sore throat and fever. ILI prevalence was high among hajj pilgrims worldwide. In 2007 hajj season, 40.1% Malaysian hajj pilgrims experienced ILI. The aim of this study is to determine the effectiveness of handrub practice in preventing ILI among Malaysian Hajj pilgrims.

METHODOLOGY

An open-label randomized controlled trial was done to Malaysian hajj pilgrims 2013 who transit in Kompleks Tabung Haji Kelana Jaya, Malaysia from 15 to 19 September 2013 before their departure for hajj. A total of 500 hajj pilgrims were recruited as study subjects (250 in control group and 250 in intervention group). The control group was given 100ml unmedicated hand lotion while intervention group was given 400mls (4 bottles) of alcohol free handrub together with validated questionnaires to record the presence of ILI symptoms and patterns of handrub usage.

RESULTS

The participants’ response rate from intervention and control group were 37.6% and 31.2% respectively. The prevalence of ILI in intervention and control group post hajj were 62.8% and 53.8% respectively and it was not statistically significant. There were reduction in percentage of frequent handrub practice (daily practice) pre-hajj versus post-hajj practice in intervention group (20.2% versus 10.6%) and in control group (24.7% versus 15.6%). Hajj pilgrims with infrequent handrub practice is significantly associated with higher incidence of ILI (p-Value 0.018, OR 3.63) compared to frequent handrub practice.

CONCLUSION

Giving handrub has not resulted in better compliance. Infrequent handrub practice associated with higher risk of getting ILI.
In-vitro antibacterial activity of bark extracts of *Rhizophora stylosa* Griff against Multispecies Oral Biofilms

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Medicinal plants have been used extensively in folklore medicine and have recently undergone rigorous investigation in preventing the various oral diseases, especially those implicated in periodontal diseases due to the formation of dental plaque. Currently available antibacterial agents in the market have poor efficacy towards the formed biofilms and hence the investigation for the new antimicrobial agents are focused on natural products. Keeping this view an attempt has been made to study the antibacterial property of *Rhizophora stylosa* bark extracts on dental biofilms by Minimum Inhibitory Concentration (MIC) and Minimum Biofilm Eradication Concentration (MBEC) by using broth microdilution method and spot plating method respectively. The bark extracts were prepared using different organic solvents in order of increasing polarity (petroleum ether, chloroform, and methanol) by using soxhlet apparatus. The biofilms were developed by seeding the various microbial consortium suspensions of varying bacterial combinations (*Streptococcus pyogenes, Streptococcus salivarius, Staphylococcus aureus* and *Pseudomonas aeruginosa*) in a 96-well flat bottom microtiter plate which were challenged over a period of 24 hours with the prepared extracts at a serially diluted concentration (200 mg/ml to 0.4 mg/ml) for MIC. The plates were read at 570nm using an ELISA microtiter plate reader. Subsequently, the MBEC was determined by spot plating the contents of the microtiter plate wells onto Tryptic Soy Agar to determine the viability of the biofilms. From the results obtained, methanol bark extracts had the best antibacterial activity, having an MIC reading of 0.4 mg/ml and an MBEC reading of 50 mg/ml when compared to the other extracts.

**Key Words:** *Rhizophora stylosa*, antibacterial activity, dental biofilms
Antibiotic resistance has been increasing drastically over the years despite the efforts to lobby against unnecessary use of antibiotics. The ability of cells to form drug resistant biofilms, a complex architecture of cells encased in an extracellular polymeric matrix, is one of the many reasons for the failure of antibacterial treatment. A classic example is the opportunistic pathogen *Pseudomonas aeruginosa* which forms biofilms on medical devices and living tissues, which is intrinsically resistant against a wide range of antibiotics. *P. aeruginosa* release virulence factors such as pyocyanin (PCY), pyochelin (PCH), pyoverdine (pvdE) and elastase (LasB) which contributes to tissue damage. We previously showed that olive leaf extract has antimicrobial activity against Gram-positive organisms. The important secoiridoids found in olive leaf such as oleuropein, hydroxytyrosol and verbascoside could play a role in the anti-microbial activity of olive leaf extracts. Therefore, this study aimed to examine the effect of olive leaf extracts and combinations of its phenolic compounds on planktonic cell growth, biofilm formation and excretion of extracellular virulence factors of *P. aeruginosa*. The effect of the extracts and its phenolic compounds on bacterial motility, which is an indication of virulence was also investigated. In general, the extracts were shown to have limited anti-microbial activity against this Gram-negative bacterium. The effects of olive leaf extracts, oleuropein, hydroxytyrosol and verbascoside on biofilm formation, virulence factors and motilities, including swimming, swarming and twitching motility are also reported.
Travel-related leptospirosis in Japan: a series of 5 imported cases at National Center for Global Health and Medicine

Background; Leptospirosis is one of the most common travel-related infections.

Methods; We retrospectively studied all the consecutive cases of travel-related leptospirosis seen in our department between January 2008 and December 2013. Patients were included with a clinical picture compatible with the disease within 21 days after return, a positive microagglutination test (MAT) or isolation of Leptospira species from a clinical specimen.

Results; 5 leptospirosis cases were evaluated. All 5 cases were contracted in Southeast Asia countries and had exposure to fresh water bathing. All 5 cases had symptoms of fever, headache, conjunctival injection, relative bradycardia. Relevant laboratory findings included elevated C-reactive protein(100%), elevated creatinine(80%), and sterile pyuria(75%). All 5 cases were diagnosed with the increase ≥4 times in MAT titer between acute phase and convalescent phase serum specimens, and 4 cases with isolation of Leptospira species from a clinical specimen in 4 cases. Patients were treated with penicillin G, minocycline, or doxycycline. 1 case were cured without antibiotics.

Conclusion; Febrile travelers returned from Southeast Asia to Japan with exposure to fresh water should be consider leptospirosis.
Fluoroquinolones are still appropriately therapeutic options for non-typhoidal Salmonella infections in southern Taiwan

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Background
Fluoroquinolone-resistant non-typhoidal Salmonella is increasing worldwide. This study was conducted to evaluate whether fluoroquinolones are still appropriately therapeutic options for non-typhoidal Salmonella infections in southern Taiwan.

Methods
This was a retrospective study at a regional hospital in southern Taiwan. From 2009 to 2013, all non-typhoidal Salmonella reported from clinical microbiology laboratory were enrolled in this study. If multiple isolates were identified from the same patient during the same hospitalization period, only the first isolate was enrolled. Antimicrobial susceptibility testing was done by disk diffusion method. The tested antibiotics included ampicillin, ceftriaxone, levofloxacin, and trimethoprim-sulfamethoxazole (TMP-SMX). The results were interpreted according to the criteria recommended by the Clinical Laboratory Standards Institute. All intermediate results were regarded as resistant in this study.

Results
A total of 122 non-typhoidal Salmonella isolates were enrolled in this study. Of the 29 isolates in 2009, the susceptibility rates of ampicillin, ceftriaxone, levofloxacin, and TMP-SMX were 69%, 96.6%, 100%, and 89.7%, respectively. Of the 17 isolates in 2010, those were 70.6%, 100%, 100%, and 88.2%, respectively. Of the 25 isolates in 2011, those were 60%, 100%, 100%, and 76%, respectively. Of the 22 isolates in 2012, those were 54.6%, 95.5%, 100%, and 90.9%, respectively. Of the 29 isolates in 2013, those were 58.6%, 100%, 100%, and 82.8%, respectively.

Conclusion
As a result of this study, levofloxacin has a susceptibility rate of 100% for non-typhoidal Salmonella during a 5-year follow-up period, indicating that fluoroquinolones are still appropriately therapeutic options for non-typhoidal Salmonella infections in southern Taiwan.
Bacterial Aetiological of Lower Respiratory Tract Infection (LRTIs) in Johor Health Clinics, 2013

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Abstract

Background: Lower respiratory tract infections (LRTIs) are among the most common infectious disease in human worldwide. Generally, these infections thought to be caused by atypical and viral infections but it also caused by bacterial infections. This study aimed to determine the bacterial aetiological that causing the LRTIs in Johor community.

Methods: A retrospective study was conducted on sputum specimens received in Johor Bahru Public Health Laboratory in 2013. A total of 2043 sputum specimens were received from patients with LRTIs attending the district health clinics. The specimens were processed according to standard methodology.

Results: Respiratory pathogens were isolated from 385 (19.5%) patients’ specimens. The major pathogens for LRTIs were found to be Klebsiella pneumoniae (52.2%), followed by Pseudomonas aeruginosa (10.7%) and Staphylococcus aureus (10.7%).

Conclusion: Gram-negative bacteria accounted for 82.3%, with Klebsiella pneumoniae as the most frequent single pathogen isolated (52.2%), from all LRTIs patients attending Johor peripheral health clinics in 2013.

Keywords: Lower respiratory tract infections, Sputum, Klebsiella pneumoniae

References


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 high 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 at 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 50mg 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.
Prevalence of acquired carbapenemases among gram negative bacteria causing neonatal sepsis
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Background: Sepsis in neonates is one of the leading causes of neonatal mortality and morbidity. Carbapenems are the choice of drug for eradication of sepsis caused by multi-drug resistant (MDR) strains of Enterobacteriaceae and Pseudomonas aeruginosa. In the past decade, an increase in the prevalence of carbapenemases and emergence of new carbapenemases has been observed. Therefore, this study was designed to determine the pattern of organisms causing neonatal sepsis and molecular analysis of the genes encoding carbapenemases. Methods: A total of 150 gram-negative strains isolated from neonates admitted in NICU were collected from Pakistan Institute of Medical Sciences, Islamabad, Pakistan. PCR analysis was carried out for the detection of genes encoding OXA-48, BIC-1, NDM-1, KPC, VIM, IMP, SPM, SIM, GIM, AIM, DIM and SIM type beta lactamases. Results: Of 150 gram negative strains, 10 were E.coli, 106 were Klebsiella pneumonia, 30 were Pseudomonas aeruginosa and 4 were Proteus spp. PCR identification revealed that 25(16.67%) strains harbored genes encoding carbapenemase. blaNDM-1 gene was found in 15(10%) isolates and blaKPC-2 gene was detected in 2 (1.8%) isolates of Klebsiella pneumoniae. blaIMP gene was found in 3.3% isolates while blaVIM gene was detected in 2% isolates. One isolate had co-existence of blaNDM-1 and blaIMP. The genes encoding BIC-1, OXA-48, SPM, SIM, GIM, AIM and DIM were not detected in any of these isolates. Conclusion: Presence of these carbapenemases in gram-negative bacteria is problematic especially in neonates, where they may lead to treatment failure. It is important to have continuous surveillance of carbapenemases in neonatal infections and strict infection control policies should be implemented in order to reduce the risk of spread of resistant pathogens.
EFFECTIVENESS OF ANDROGRAHIS PANICULATA AND TINOSPORA CRISPA AS INHIBITORS FOR TOXOPLASMA GONDII TACHYZOITES

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ABSTRACT

Toxoplasma gondii is an intracellular apicomplexan protozoan parasite related to the causative agents of coccidiosis (Eimeria spp). It is found world-wide, at high prevalence and in an exceptionally broad host range, making it one of the most ‘successful’ parasite on earth. The antitoxoplasmic activity of A. paniculata and T. crispa were evaluated in murine models of intraperitoneal infection using the RH strain of Toxoplasma gondii. In mice infected with 2x10^2 tachyzoites/ml, treatment with those plant extracts at 100 and 200 mg/kg/day had a good effect. Administration of tested extracts intraperitoneally reduced the mean number of tachyzoites present in the peritoneal fluid of experimented mice 4 days after infection, with the treatment of 100 and 200 mg/kg/day of plant extracts the percentage of growth inhibitions ranged from 96.6% to 100%, as that of the reference drug, Spiramycin, (% growth inhibitions were 96.6%, 75.9% correspondingly) compared by the control infected untreated mice. Spiramycin has proved to be helpful drug used at present and it may be practical if its dose was higher than those used in this study. Regarding the results recorded here, the plant extracts used as antitoxoplasmosis drugs proved to be of considerable effect. To be convinced for these plants effect as useful drugs against T. gondii tachyzoites, also it be supposed to be examined in vitro as well as antitoxoplasmosis drugs.

Key words:
Antitoxoplastic Activity, Spiramycin, Toxoplasma Gondii, Growth Inhibition
The prevalence of *Pseudomonas aeruginosa* Producing blaIMP1, blaVIM2, blaSIM1, blaSPM1 isolates in Shiraz, Iran

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**Background:** Metallo-β-lactamase (MBL) producing *Pseudomonas aeruginosa* is responsible for many important nosocomial outbreaks including pneumonia and septicemia. Various studies have reported the separation of *P. aeruginosa* producing MBLs resulted in increases to multiple traditional antibiotics resistance including carbapenems, cephalosporins and penicillins. In this study, based on the standard phenotypic and genotypic methods, we have examined the MBLs possible production of an *P. aeruginosa* isolates. The main objective of this study was to explore the dissemination of resistant MBL *P. aeruginosa* in Shiraz, southwest Iran.

**Methods:** During a six-month period— from October 2011 to March 2012— 240 isolates of *P. aeruginosa* were collected from four teaching hospitals in Shiraz, Iran, were examined. The isolates were mainly collected from wound, urine, and sputum. Minimum inhibitory concentration (MIC) ≥ 4 µg/mL to imipenem was determined by micro-dilution broth. Identification of *P. aeruginosa* with MBL was detected using double disk synergy test (DDST) and polymerase chain reaction (PCR) using specific primers for blaIMP1, blaVIM2, blaSIM1, blaSPM1. All laboratory procedures were according to clinical and laboratory standards institute (CLSI) recommendations.

**Results:** From 240 *P. aeruginosa* isolates, 82 (34.16%) isolates were imipenem-resistant (minimum inhibitory concentration (MIC) ≥ 4 µg/mL). Among these imipenem-resistant isolates, 19 (23.3%) MBL-producing *P. aeruginosa* isolates were screened using DDST. A specific PCR test confirmed the presence of 18 (21.95%) isolates of *P. aeruginosa* producing blaIMP1 and blaVIM2.

**Conclusions:** Beside our study, the detection of MBL genes were reported in a few studies conducted in Iran. The spread of detected MBLs producing *P. aeruginosa* were unprecedented in the region due to the lack of independent related researches or the novel incidence of these genes. This detection must be noted by associated clinical and health care services.
Investigation of nasal *Staphylococcus aureus* carriage in food handlers

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BACKGROUND: *Staphylococci* are a group of bacteria that may cause serious infections in humans. They may colonize skin mostly nares and may be transmitted by droplets containing bacteria and by direct contact. Nasal *Staphylococcus aureus* carriage is an important risk factor that can lead to infections. This survey is conducted between January 2010 and January 2014, on food handlers admitted for mandatory screening tests.

METHODS: Nasal sampling was performed with sterile cotton tipped swabs from both nostrils. Swabs are inoculated onto 5% sheep blood agar and incubated at 37°C for 24-48 hours. Beta hemolytic yellow colonies on sheep blood agar are Gram stained and observed at x1000 magnification. Gram positive cocci giving positive results with catalase, tube coagulase and DNAse test are assumed as *S. aureus*. Methicillin resistance is tested with modified Kirby-Bauer disk diffusion test according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

RESULTS: The total number of 4487 food handlers was evaluated with culture results. From 4487 cultures studied, 616 (13.7%) cultures yielded *S. aureus*. Among 616 *S. aureus* isolates, 151 (24.5%) were identified as methicillin resistant *S. aureus* (MRSA), 465 (75.5%) were methicillin susceptible *S. aureus* (MSSA).

CONCLUSION: *S. aureus* nasal carriage possesses a key role in epidemiology and pathogenesis of infections related with this pathogen, particularly foodborne diseases in this case. Hands are often contaminated with *S. aureus* in nasal carriers and poorly washed or sanitized hands can easily contaminate food. Actually staphylococcal food poisoning is caused by eating foods contaminated with toxins produced by *S. aureus*. In brief, periodical screening of food handlers and assigning personnel, according to these results, will decrease the number of food poisoning cases related with *S. aureus*. 
Cervical Lymphadenitis due to Toxoplasmosis: A case Report
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BACKGROUND: Toxoplasma gondii is a widespread intracellular parasite that affects all the mammals including humans. While cats are the only definitive hosts, other mammals are intermediate hosts for T. gondii. People become infected by eating undercooked or raw meat, and directly ingesting the oocysts in contaminated water or foods with cat feces. Toxoplasmosis, a common zoonotic infection caused by T. gondii, mostly is not symptomatic in immunocompetent hosts, but in immunosuppressive hosts it can cause severe infections affecting many systems and organs. Various tests can be used in diagnosis, but ELISA is the most preferred method in many laboratories since it is economic, reliable and easy to use.

METHODS: A soft and mobile mass in right side of the neck of a 56 years old woman is removed for pathologic examination by excisional biopsy. It had smooth margins, rubbery consistency, homogeneous appearance in cross-sections, and it had dimensions of 1.8 cm x 1 cm x 0.5 cm.

RESULTS: Reactive follicular hyperplasia was observed in H/E stained sections of lymph node. Germinal center formation was obvious, tingible body macrophages were increased and alsohistiocytes were increased around the sinuses and blood vessels in the lymph node. Numerous toxoplasma bradizoites were seen in cytoplasm of histiocytes in PAS stained examination. Upon these findings new tests were requested. Toxoplasma antibodies were investigated with ARCHITECT Toxo IgM and IgG assays (Abbott Laboratories, Wiesbaden, Germany). Toxoplasma IgM was negative, toxoplasma IgG was positive and complete blood count test wasn’t indicating any pathologic finding. In addition, the patient didn’t have any other disorders except toxoplasma lymphadenitis.

CONCLUSION: While toxoplasmosis is a common parasitic infection, in 90% of the immunocompetent hosts it is asymptomatic and in the remaining 10% it causes a mild and self-limited disease. Most common finding is asymptomatic lymphadenopathy. Lymph nodes located in posterior cervical triangle of the neck are the most affected group. Most obvious pathologic finding is the conservation of the general structure and observation of epithelioid histiocyte groups that is important for toxoplasmosis. Diagnosis of toxoplasmosis is basically made with serologic tests. Histopathologic examination of the cervical lymph nodes revealed toxoplasma lymphadenitis. Additional diagnostic tests such as PCR with T. gondii genome specific primers may be used to improve diagnosis.
The effect of antifungal combination on transcripts of a subset of drug-resistance genes in clinical isolates of *Candida* species induced biofilms

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Abstract

Biofilm formation is often associated with increased *Candida* resistance toward antifungal agents. Therefore, the current study aimed to assess the incidence of biofilm formation among *Candida* isolates and to investigate the effect of high doses of fluconazole (FLC), voriconazole (VOC) and amphotericin B (AMB), singly and in combination on mature biofilms. Moreover, it aimed to assess the expression of selected genes (CDR1, KRE1 and SKN1) responsible for *Candida* biofilm resistance. The study included 49 patients; samples were collected from the King Khalid Hospital, Riyadh, Saudi Arabia. Isolates were prepared for biofilm formation and quantification using 0.4% (w/v) crystal violet. Minimum Inhibitory concentration (MIC) and fractional inhibitory concentration (FIC) were conducted by the broth microdilution method. Biofilm eradication was evaluated using counting, XTT stain intensity and observed under the inverted microscope. Selected genes were evaluated in *Candida* biofilms under the effect of antifungal exposure using QPCR. The major isolates were *Candida albicans* (65.3%) followed by *Candida tropicalis* and *Candida glabrata*. 77.6% of the strains were biofilm formers. AMB showed susceptibility in 87.8% of isolates, followed by VOC (77.6%) and FLC (67.3%). MIC50 and MIC90 were (0.03, 0.125), (0.5, 8), (2, >128) μg/ml for AMB, VOC and FLC, respectively. 34.7% and 18.4% of the isolates were antagonistic to AMB/FLC and AMB/VOC, respectively. Mature biofilms of ten selected isolates were found resistant to FLC (1000 μg/ml). VOR and AMB concentration required to inhibit biofilm formation was 16–250 fold higher than the MIC for planktonic cells. Isolates showed significant reduction with antifungal combination when compared with the untreated controls (p value < 0.01), or using fluconazole alone (p value < 0.05). High doses of the antifungals were employed to assess the effect on the persisters’ selected gene expression. Marked over expression of SKN1 and to a lesser extent KRE1 was noticed among the mature biofilms treated with AMB alone or in combination after 1 h of exposure, and SKN1 expression was even more sharply induced after 24 h. No statistically significant over expression of CDR1 was observed in biofilms after exposure to high doses of FLC, VOC or any of the combinations used.

Biography

Nermin is an Assistant Professor of Microbiology and Immunology, School of Pharmacy, king Saud University. She received her M.D. in Medical Microbiology from the Faculty of Medicine Cairo University, Egypt (2006), followed by many training courses and workshops in molecular biology, mycology and infection control programs. She worked as a teacher assistant, lecturer in Department of Medical Microbiology and Immunology at Faculty of Medicine, Cairo University till 2006, then, as lecturer and Assistant Professor at Medicine school, BeniSuef university till 2009. She is amember of the Egyptian Society of Medical Microbiology, Society of Immunology and Allergy and Arab Alliance for the Prudent Use of Antimicrobials. Her main area of specialty and interest include virulence factors of bacteria and fungi especially biofilms, and studying their genetic diversity.
Cross-sectional study on ESBL prevalence of urinary Enterobacteriaceae among children in a District General Hospital in North Western Sri Lanka
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Background
Extended spectrum beta-lactamase (ESBL) production is an important resistance impeding treatment of infections caused by Enterobacteriaceae. Sometimes ESBL resistance carries resistance to non beta-lactam antibiotics in addition to beta-lactams limiting the choice of antibiotics. ESBL production among uropathogens is increasing and prevalence varies between hospitals. In children, appropriate treatment of urinary tract infections (UTI) prevents pyelonephritis, urosepsis and late sequelae such as renal scarring or renal failure. Hence, knowledge of ESBL prevalence is important in treating UTI in children.

Objectives
To calculate ESBL prevalence from 2011-2013 and in different age groups
To determine the non-beta-lactam resistance of ESBL producing Enterobacteriaceae

Method
This cross-sectional study was carried out of the records from January 2011 to September 2013 in the Microbiology Department of a District General Hospital in Sri Lanka. Standard isolation and identification was carried out. Antibiotic susceptibility test was by disk diffusion method. Disk approximation test using clavulanic acid was done to identify ESBL production. Lab records of all significant urine cultures of patients below 12 years of age were reviewed. Chi-Square as well as Fisher's exact test was used to compare categorical variables using SPSS 13 for Windows®. P value < 0.05 was considered as statistically significant.

Results
During the study period 2604 urinary cultures of patients below 12 years of age were processed. 421 isolates were of Enterobacteriaceae with 60% in males. Among Enterobacteriaceae, 47% (196) were from infants, 33% (139) from 1 to 5 years children and 20% (86) from 5 to 12 years olds. Overall ESBL rates for 2011, 2012 and 2013 were 10.6% (16/150), 21.4% (35/163) and 19.4% (21/108) respectively. ESBL rate was highest in infants (38.7%) followed by 35.7% for 1 to 5 years and 17.3% for 5 to 12 years.

The proportion of ESBL-producing Enterobacteriaceae resistant to antibiotics was 82% for nalidixic acid, 73% for norfloxacin, 68% for trimethoprim-sulfamethoxazole, 64% for ciprofloxacin and 58% for gentamicin. For non-ESBL-producing Enterobacteriaceae, resistance rates were 39%, 23%, 45%, 13% and 5% in that order. There was no significant difference in resistance for non-ESBL and ESBL producing Enterobacteriaceae to nitrofurantoin (29% vs 21%) amikacin (8% vs 6%) and meropenam (4% vs 3%). Multi-drug resistance among ESBL producers and non-ESBL producers was 53% and 12% respectively.

Conclusion
This study highlights an increasing high ESBL prevalence in children especially in under 5 years of age. High multi-drug resistance among ESBL producers is alarming. Local susceptibility and ESBL prevalence should be considered in treatment.
**Distribution of *Helicobacter pylori* virulence genes in Malaysia**

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**Abstract**

**Background:** Several virulence factors of *Helicobacter pylori* have been reported to be predictors of severe clinical outcome. The aim of this study is to determine the distribution of sabA, babA2 and dupA and its clinical significance.

**Methods:** This is a cross sectional study conducted between July 2012 to January 2014 among 226 dyspeptic patients at the Endoscopy Unit of Hospital Universiti Sains Malaysia and Hospital Kuala Lumpur. *H. pylori* sabA, babA2 and dupA genes were genotyped by polymerase chain reaction (PCR). Chi-square and Fisher’s exact test was used in statistical analysis.

**Results:** Out of 226 patients, 105 (46.5%) were confirmed *H. pylori* positive. There were 57 (54.3%) males and 48 (45.7%) females. The distribution of sabA, babA2 and dupA genes in *H. pylori* positive patients was 43.8%, 41.0%, and 22.9% respectively. sabA and babA2 is more common in Malaya at a rate of 43.5% and 39.5% respectively, while dupA detection is same in both Indian and Malay (37.5%). The Chinese have the lowest distribution of the three virulence genes. Based on the endoscopic findings, 56 patients had non ulcer dyspepsia (NUD) or gastritis, 8 gastric ulcer, 3 duodenal ulcer and 6 normal.

**Conclusion:** our results showed the variation in the distribution of *H. pylori* sabA, babA2 and dupA genes in Malaysia among *H. pylori* positive patients. There was no significant association between sabA, babA2 and dupA genes and the clinical outcome in our study.
Frequency of human parvovirus 4 among HBV-infected patients and healthy donors in Shiraz, Iran

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\textbf{Background:} Human parvovirus 4 (PARV4) is a small DNA virus belonging to the \textit{Parvoviridae} family. PARV4 was first isolated in 2005 in a HBV injecting drug user (IDU) and several studies have investigated PARV4 co-infection with HBV and HCV and its effect on the progression of liver disease. So far, three PARV4 genotypes have been identified, however, with an unknown clinical significance. PARV4 is detected from various samples such as plasma from healthy donors; serum and liver biopsy from HBV and HCV patients; serum, bone marrow and lymphoid tissue from people living with HIV. This study was aimed to determine the frequency of PARV4 among HBV-infected patients and healthy individuals.

\textbf{Methods:} A group of 90 HBV patients who were negative for HCV and HIV and a group of 90 healthy subjects were included in this study. These 180 samples were selected after screening tests such as HBs Ag ELISA, HCV Ab ELISA, and HIV Ab ELISA. Nested-PCR was conducted to detect PARV4 genome. Positive samples were then subjected to DNA sequencing.

\textbf{Results:} PARV4 DNA was detected in 4 out of 90 (4.4%) patients with hepatitis B in comparison with only 1 out of 90 (1.1%) healthy individuals (p value: 0.04). Sequencing results revealed that PARV4 in all five positive samples were PARV4 genotype 1.

\textbf{Conclusion:} Although this pilot study showed higher frequency of PARV4 among HBV patients, further studies with a larger sample size is suggested in order to determine the impact of this virus on the progression of liver disease in patients with hepatitis B.

\textbf{Keywords:} PARV4, \textit{Parvoviridae}, HBV, Molecular detection, Genotyping
Whole genome analyses of an extensive drug-resistant clinical isolate of *Acinetobacter baumannii* AC12 from Terengganu, Malaysia reveals the presence of three antibiotic resistance islands

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Background: *Acinetobacter baumannii* has been implicated in various nosocomial infections and the therapeutic option for these *Acinetobacter* infections is increasingly limited due to the uncanny ability of the bacterium to acquire antimicrobial resistance determinants. These determinants are sometimes found clustered together in mobile regions of the genome known as resistance islands (RIs). An extensive drug resistant (XDR) *A. baumannii* strain AC12 was isolated from the blood of a male patient who was warded in a tertiary hospital in Terengganu, Malaysia in 2011. Here, we present the whole genome sequence analyses of *A. baumannii* AC12 that led to the discovery of three resistance islands in this strain.

Methods: The genome of *A. baumannii* AC12 was completely sequenced using Illumina genome analyzer IIx. The resulting paired-end reads were trimmed and assembled using CLCBio Genomics Workbench 5.0. Then, gene prediction was carried out using Prodigal 2.60 followed by annotation using Blast2Go and the RAST annotation server. The sequence type (ST) of *A. baumannii*AC12 was determined by accessing the PubMLST database. Comparative genome analyses were further carried out to elucidate the genetic basis of its antimicrobial resistance. The *A. baumannii* AC12 genome sequence has been deposited in GenBank under accession number CP007549.

Results: The *A. baumannii* AC12 genome consists of a 3.8 Mbp circular chromosome and an 8,731 bp cryptic plasmid, pAC12. It belongs to the ST195 lineage and is most closely related to *A. baumannii* BJAB0715 as well as other strains of the International Clone III (IC-III) group. Two major antibiotic resistance islands (RIs), designated AC12-R11 and AC12-R12, were found in the AC12 chromosome. The 22.8 kb AC12-R11 interrupts the *comM* gene and harbours the carbapenem resistance gene *bla*OXA-23 flanked by IS*Aba*1 within a Tn2006-like structure. AC12-R11 also contains resistance determinants for aminoglycosides, tetracyclines and sulphonamides. The 10.3 kb IS26-flanked AC12-R12 is a derivative of AbGRI2-1 and contained *aphA1b* and *bla*TEM genes which confer aminoglycoside and β-lactam resistance, respectively. *A. baumannii*AC12 also harboured a 7 kb Tn1548::armA island that encode determinants for aminoglycoside and macrolide resistance and was previously found in plasmids of *A. baumannii* and several *Enterobacteriaceae*.

Conclusions: The presence of numerous genes mediating resistance to various antibiotics in novel resistance island structures in the *A. baumannii* AC12 genome contributed to its extensive drug-resistance characteristics.

(446 Words)
MOLECULAR EPIDEMIOLOGY OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATED FROM A MALAYSIAN UNIVERSITY HOSPITAL

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Background: MRSA is an important pathogen associated with nosocomial infections. We determined the molecular epidemiology of MRSA strains isolated from Universiti Kebangsaan Malaysia Medical Centre (UKMMC).

Materials & Methods: The first strain isolated from each MRSA infections in 2009 at UKMMC was collected. DNA of MRSA strains were extracted and molecularly typed by Pulsed-Field Gel Electrophoresis (PFGE).

Results: A total of 318 cases of MRSA infection were recorded in UKMMC in 2009. PFGE typing on 318 MRSA isolates identified 27 pulsotypes (A to AA) where 4 of them (F, N, Q and T) appeared to be the predominant pulsotypes in UKMMC. There are significant association (p=0.00) between 4 dominant PFGE pulsotypes with the medical, surgical and others (PICU, CCU, NICU and ICU) ward. Majority of the MRSA strains in pulsotype F were isolated from medical ward (45.9%), 24.6% of MRSA strains were from surgical ward and 29.5% were from others ward. Majority of the MRSA strains in pulsotype N were isolated from surgical ward (66.7%) whereas 33.3% were from medical ward. None of them were detected in others ward. Majority of the MRSA strains in pulsotype Q and T were detected in surgical ward 100% and 90.9% respectively.

Conclusion: There was a different dominant pulsotype of MRSA in different wards in UKMMC; with pulsotype F MRSA circulating in all wards. This finding is useful in establishment of appropriate preventive measures against the transmission of MRSA infections.
Clinical features of clarithromycin-resistant nontuberculous mycobacterial diseases in Nagasaki, Japan


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Background: The prevalence of nontuberculous mycobacterial (NTM) diseases is increasing in Japan. Clarithromycin (CAM) is the mainstay drug of treatment regimens for *Mycobacterium avium* complex (MAC) and the development of CAM-resistance is known as an adverse prognostic factor in MAC. The objectives of the current study are to assess the characteristics of the patients infected with CAM-resistant NTM.

Methods: Retrospective chart review, susceptibility testing of CAM-resistant NTM isolates obtained from 157 consecutive patients attended Nagasaki University Hospital between June 2008 and December 2012 were performed. Results: We identified 35 patients (male/female ratio; 13/22) with CAM-resistant NTM (minimum inhibitory concentration (MIC) ≥ 32μg/ml) diseases at a single referral center. The mean age at the onset of NTM disease was 63.3±14.7 years (male; 64.3±15.2 years, female; 62.7±14.4 years). Twenty-one (60%) and eight (23%) patients had *M. avium* and *M. intracellulare*, respectively. Twenty-seven (77%) patients had underlying pulmonary diseases. Sixteen (46%) patients had nodular disease with bronchiectasis and twelve (34%) patients had fibrocavitary disease. Radiological changes 1 year after diagnosis were as follows: improve: thirteen (37%) patients, progress: nine (26%) patients, stable: three (8%) patients, not applicable: ten (29%) patients. All of patients showing the radiological improvement were treated with multi-drug regimen with CAM and nine of thirteen (69%) patients were administered higher dosing of CAM (more than 800mg/day) whereas eighteen of twenty-two (82%) patients shown no radiological improvement were treated with CAM under 600mg/day or the regimen without CAM. The 1-year sputum culture conversion of CAM-resistant NTM treated with higher dosing of CAM was ten of fourteen (71%) patients, in contrast the sputum conversion of the patients treated with CAM under 600mg/day was only two of eight (25%). Conclusion: These results suggested that CAM-resistant NTM disease might require aggressive drug such as multi-drug chemotherapy with higher dosing of CAM (more than 800mg/day) for cure.
Z-RNA binding confers protein localization to cytoplasmic stress granules and is implicated in antiviral response

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DNA normally adopts the B-form right-handed double helical structure under physiological conditions. However, DNA can also adopt the higher-energy, left-handed, Z-form under high salt conditions. Alternating purine:pyrimidine DNA sequences, such as (dG:dC)ₙ were found to be more favorable to adopting Z-form. Subsequently, an attempt to identify proteins with a high affinity for Z-DNA revealed the presence of Z-DNA binding domains (ZBDs) in RNA-editing enzyme ADAR1. ZBDs has also been identified in four other proteins: ZBP1, poxvirus E3L, fish protein PKZ, and cyprinid herpervirus3 ORF112. Crystal structures of the Za domain of these proteins revealed a conserved protein-DNA interactions along the sugar-phosphate backbone, specific to the Z-DNA. Double-stranded RNA can also adopt the Z-form (Z-RNA), and binds to the Za domain of ADAR1 in a similar manner to Z-DNA. While Z-DNA can form at transcription sites, the physiological role of Z-DNA/Z-RNA is not well understood.

ADAR1 has two major isoforms from alternative transcription start site; a constitutively expressed smaller isoform ADAR1⁰¹⁰ in the nucleus, and an interferon-inducible larger isoform ADAR1⁰¹⁵ that is mainly cytoplasmic. ADAR1⁰¹⁵ localizes to cytoplasmic stress granules induced by arsenite treatment or poly(IC) transfection. Further studies using immunofluorescence revealed that the ZaADAR1 domain was necessary and sufficient for localization to stress granules in HeLa cells. In addition, the attachment of the ZaADAR1 domain to another protein (GFP or PTB4) was sufficient for localization to stress granules. Mutagenesis studies then confirmed that Z-DNA/Z-RNA binding of the Za domain was necessary for the localization of ADAR1⁰¹⁵ to stress granules. Both of the other ZBD-containing proteins tested, ZBP1 and E3L also showed that their respective Za domains were sufficient for localization to stress granules. Although not tested, the Za domains of PKZ and ORF112 are likely to have the same function given the conservation of Za domains among these five ZBD-containing proteins.

We thus discovered a novel cellular function of the Za domain. Cytoplasmic stress granules are formed following cellular stress to promote cell survival. It has not escaped our attention that all five ZBD-containing proteins are involved in viral-host relationship. Both ADAR1 and ZBP1 are known effectors during interferon response, where ZBP1 is a cytosolic DNA sensor. Meanwhile, E3L is essential for vaccinia virus pathogenesis in mouse model. The interaction of these ZBD-containing proteins in stress granules require further investigation, and may contribute to the understanding of interferon response. Moreover, the role of (presumably) Z-RNA in cytoplasmic stress granules also remains to be elucidated.
Isolation and Identification of Pathogenic Yeasts from Fruit Surfaces

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Infections caused by treatment-resistant non-albicans Candida species, such as C. tropicalis, have increased, which is an emerging challenge in managing fungal infections. Previous studies have isolated C. tropicalis of the same diploid sequence type (DST) from human and soils. However, the routes of spreading remain elusive. In the present study, we isolated and characterized pathogenic yeasts on the surface of fruits from supermarkets. 291 isolates of 83 species from 24 different types of fruits were recovered. Of the 83 species, 7 common pathogenic Candida species were detected. They included 16 C. guilliermondii, 15 C. famata, 3 each of C. parapsilosis and C. tropicalis, 2 each of C. krusei, C. lusitaniae, and C. orthopsilosis. The drug susceptibilities of 162 of the 291 isolates were determined. Totally, 158 (97.5%), 104 (64.2%), and 102 (63%) isolates were susceptible to amphotericin B (MICs ≤ 4 mg/L), fluconazole (MICs ≤ 8 mg/L), and triadimenol (MICs ≤ 8 mg/L), respectively. One C. tropicalis isolate (F91) from wax apple had MICs at 64 mg/L for both fluconazole and triadimenol. It belongs to DST 149, a genotype found in isolates from human as well as soil. Thus, extra caution shall be taken when providing fruits or juice to severely immunocompromised patients since drug resistant pathogenic yeasts may be on the surface of fruits.
Heightened protease activity in ST 3 *Blastocystis*.

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*Blastocystis* sp. is the most frequently isolated parasite in any stool survey carried out globally. There have been up to 17 subtypes (ST) reported with ST 3 incriminated to be the pathogenic one. Although protease activity in *Blastocystis* sp. has been shown to contribute to pathogenesis, their correlation to different STs’ and their respective specific type of protease remains to be elucidated. Both the lysates and excretory and secretory product (ES products) from ST1, ST3, ST 5 and ST 6 belonging to symptomatic and asymptomatic individuals were isolated and subjected for detection and quantification of protease. Inhibition assay involving different protease inhibitor was carried out to determine specific protease in each ST. The present study showed that protease activity from ST 3 to be 2 to 3 fold higher compared to other subtypes. Protease activity from all symptomatic isolates was significantly higher compared to asymptomatic isolates. All the STs showed highly significant (p<0.05) inhibition towards cysteine protease-specific inhibitor (E64) suggesting the predominance of cysteine protease in all STs with varying amount. This study for the first time has demonstrated a heightened protease activity with respect to different ST suggesting the genotypic variability in pathogenicity. Elevated protease activity in ST 3 explains the pathogenic potential of this ST as previously reported.
Title: Can thermal stress induce proliferation of Blastocystis sp.?

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Abstract:

Blastocystis sp. is known to be the most commonly found intestinal protozoan parasite in human and has been incriminated to be responsible for diarrhea and abdominal bloating. Binary fission has been widely accepted as the plausible mode of replication for this parasite. However, there were other modes of replication which has been suggested including schizogony, budding and sac-like pouches which produce progenies of Blastocystis sp. The present study demonstrates for the first time the role of thermal stress in proliferation of Blastocystis sp. The proliferation of Blastocystis was assessed by subjecting cultures to high temperature (41°C). The parasite growth was reduced to a minimal with a majority of the organism showing granular forms after 24 hours exposure to 41°C. The growth however doubled compared to the control isolates when the parasites were re-cultured back at 37°C. Upon thermal stress at 41°C, subtype 3 and subtype 5 isolates’ growth reached up to 2.97×10⁶ and 3.05×10⁶ cells/ml compared to their respective controlled culture tubes at 37°C which peaked only at 1.34×10⁶ and 1.70×10⁶ cells/ml respectively. Besides, the thermal stressed cultures were also analyzed using acridine orange staining. They have higher green fluorescence intensity showing presence of DNA at the central body compared to the control ones implying that there were more granular forms. This proved that the thermal stress induce formation of reproductive granular forms with viable granules in the central body. This study also provided for the first time the TEM pictures of thermal stressed Blastocystis sp. The thermal stressed Blastocystis has central body filled with highly electron dense material containing tiny granules. This again provides evidence for the formation of reproductive granular forms containing granules under thermal stress which has the ability to produce viable daughter cells. Higher number of granular forms was significantly found in thermal stressed isolates of subtype 3 and 5 which implicates that thermal stress has a role in the life cycle of Blastocystis sp.
The Analysis of Distribution of Multidrug resistant *Pseudomonas* and *Bacillus* species from Burn Patients and Burn Ward Environment

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**ABSTRACT**

Introduction: Infections caused by multidrug resistant bacteria act as a risk factor for mortality in burns patients. So keeping in view the crucial importance of reliable therapeutic decisions of multidrug resistance bacteria and routes of bacteria colonization, our study is based on the evaluation of distribution of *Pseudomonas* sp. and *Bacillus* sp. in burn patients and burn ward environment.

Methods: The present prospective analysis was conducted on the patients undergoing treatment in the Burn ward of Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana, during the period of January 2012 to March 2013. The multidrug resistant bacteria were characterized by following the CLSI guidelines. For molecular epidemiology identification gene coding for 16S rDNA was amplified.

Results: In our study out of 510 samples of 280 burn patients, 263 samples were observed sterile and bacterial isolates were obtained from 247 samples. In burn patients out of 247 samples 43 MDR strains, and in burn ward out of 60 samples 4 MDR strain were observed. After 16S rDNA amplification of MDR isolates the prevalent bacterium was belonged to the genus *Bacillus* (8 Species; 26 isolates) followed by genus *Pseudomonas* (5 species; 17 isolates). The burn ward environment isolates were belonged to *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Bacillus cereus* and *Acinetobacter baumannii*.

Conclusion: So major finding of our study is the predominance of *B. cereus* followed by *P. aeruginosa* in burn patients of Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana. The major reason of infection in burn patients was cross infection not through the hospital environment.
The opportunistic role of a travel clinic
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Global travel is associated with the importation of infectious diseases across borders. Vaccination is the most cost-effective preventive tool against travel-related diseases and many community-acquired infections. We report on our travellers’ epidemiology and their uptake of vaccine advice from a single outpatient travel clinic in a large adult tertiary hospital.

Methods: Retrospective review of case records of attendees at the travel clinic in Singapore General Hospital from 29 Oct 2010 to 4 April 2014.

Results: During this period, 3011 persons attended our travel clinic. Two thousand eight hundred and six (93.2%) persons received one or more vaccines, and the remaining 205 received only travel advice and/or prescriptions of anti-malarial prophylaxis medication. Among all attendees, 54% were women and predominantly between 20 and 40 years old (51%). The main purpose for attendance at the travel clinic was for pre-travel consultation (91.4%); other reasons were for completion of scheduled vaccination, as part of transplant recipient immunisation program or for occupational vaccination requirements. The three commonest travel destinations recorded were Asia (52.0%), Africa (19.0%) and South America (13.0%).

A total of 5091 vaccines were administered. The top five vaccines administered were for Typhoid (23.8%), Hepatitis A (17.0%), Yellow fever (14.6%), Influenza (14.3%), and Tetanus, diphtheria and pertussis (8.3%). Seven hundred and forty-three attendees received yellow fever vaccine, amongst which, 60 (8.1%) were above the age of 60. Eleven of the elderly yellow fever vaccine recipients were above the age of 70. There were no reported cases of yellow fever vaccine-associated viscerotropic or neurologic disease during this study period.

Besides travel-related vaccinations, 724 (14.2%) vaccinations administered were considered as booster shots of routine vaccinations including Measles Mumps, Rubella (MMR), Tetanus, diphtheria and pertussis (Tdap), Poliomyelitis and Hepatitis B.

Conclusion: Most of the travel clinic attendance was pre-travel consultation with Asia being the most popular destination. Typhoid and Hepatitis A vaccines were the commonest vaccines administered. Despite an increased risk of vaccine-associated viscerotropic or neurologic reactions in older age group, the majority of the elderly travellers who attended our clinic for yellow fever vaccine advice, opted to receive the vaccine. None experienced any significant adverse events. The travel consult also provided an opportunity to update the travellers’ routine vaccinations. This can be particularly helpful for the healthy adult, who does not routinely visit healthcare providers. In conclusion, travel clinics are not only dedicated to provide travel health advice and vaccinations, but can also serve as important platforms to update one’s immunisation status.
Antibacterial Resistance Among *Streptococcus pneumoniae* and *Haemophilus influenzae* from Malaysia: Results from the Survey of Antibiotic Resistance (SOAR) 2009-2011.

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**Objectives:** Antibacterial resistance among community-acquired respiratory tract pathogens such as *Streptococcus pneumoniae* (SP) and *Haemophilus influenzae* (HI) is a problem in many parts of the world and especially in Asia. SP and HI data from the SOAR programme in Malaysia are reported.

**Methods:** During 2009 to 2011, 99 SP and 90 HI were collected from Malaysia. MICs for various antimicrobials were determined using Etest or disk susceptibility testing (according to CLSI guidelines). Susceptibility was assessed using CLSI interpretive criteria except for clarithromycin where bioMerieux Etest breakpoints in CO₂ were used.

**Results:** Overall, 80.8% (80/99) of SP were penicillin-susceptible and 19.2% (19/99) were penicillin-non-susceptible based on CLSI oral penicillin breakpoints. Using CLSI IV penicillin breakpoints, 92.9% (92/99) were susceptible to penicillin. Overall, susceptibility to levofloxacin, amoxicillin/clavulanic acid, ceftriaxone, clarithromycin, trimethoprim-sulphamethoxazole, and tetracycline was 100% (99/99), 98.0% (97/99), 97.0% (96/99), 77.8% (77/99), 65.7% (65/99) and 28.3% (28/99), respectively. Based on PK/PD breakpoints, 72.7% (72/99) were susceptible to ciprofloxacin. For HI, 12.0% (11/90) produced beta-lactamase and 1 isolate (1.0%) was beta-lactamase-negative but ampicillin resistant (BLNAR). All HI were fully susceptible to amoxicillin/clavulanic acid, ceftriaxone, chloramphenicol and levofloxacin. Susceptibility was also high to cefuroxime and clarithromycin at 98.9% (89/90) and 97.8% (88/90), respectively.

**Conclusions:** Among SP strains, levofloxacin, amoxicillin/clavulanic acid and ceftriaxone were the most active antibacterials but there was evidence of some penicillin non-susceptibility and lower susceptibility was observed for clarithromycin, trimethoprim-sulphamethoxazole and tetracycline. Among HI strains most agents retained very good *in vitro* activity but beta-lactamases were detected in 12.0% of isolates. Although antibacterial resistance was found to be relatively low in Malaysia continued surveillance is necessary to track any changes in susceptibility over time.

Allowed 450 words (to include title, author names & affiliations).
Carbapenem Prescribing Survey And Its impact On The Usage Of Carbapenems In A Private Hospital Setting

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Background: According to the Annual Antibiotic Usage data submitted to the Ministry of Health (MOH) Malaysia in 2012, Sunway Medical Centre (SunMed) had shown a high usage of meropenem compared to other private hospitals in the survey. The aim of this study was to identify the reasons for prescribing carbapenems (meropenem, imipenem and doripenem) and to determine its impact on the usage of carbapenems in SunMed.

Method: This cross-sectional study was conducted between 1st September 2013 to 28th February 2014. All admitted patients who were prescribed carbapenems were included in this study. Each prescriber was given a standardized survey form with a list of reasons for prescribing (see Figure 1) to be filled up accordingly. Data on culture and sensitivity (C&S) testing results (if any) and the subsequent change in therapy after C&S results obtained were compiled for tracking, trending, and analysis. The usage of carbapenems were compared before, during and after the survey period and expressed in terms of Defined Daily Dose (DDD) per 100 admissions.

Results: Out of 178 cases initiated with a carbapenem, 139 (78%) had survey forms filled. The various reasons for prescribing carbapenems are illustrated in Figure 1. Out of 139 cases, 127 cases (91%) had cultures taken prior to initiating a carbapenem and 53 cases (38%) had positive microbial growth. Of these 127 cases, 99 (78%) cases were continued on carbapenems, 23 (18%) cases had their carbapenems changed to other antimicrobials, and 5 (4%) cases had their carbapenems discontinued with no alternative antimicrobials prescribed. It was observed that the DDD per 100 admissions of both meropenem and imipenem had a marked reduction of 22.89% and 55.06% respectively in 2013 compared to 2012. After the study had completed, the DDD per 100 admissions for carbapenems reduced further by 14.35% in the first half of 2014.

Conclusion: This study enabled us to identify the various reasons for prescribing carbapenems and its impact on the usage of carbapenems in SunMed. We observed a decline in the usage of these antibiotics during and soon after the study. The data obtained provided valuable insight into the prescribing behavior of carbapenems among the clinicians that may assist us in the development of future antibiotic stewardship initiatives.

Figure 1: The reason(s) for prescribing carbapenems.
Synergistic Effects of New Gentamicin-\textit{N.sativa} Emulsion Against Gentamicin-resistant, Biofilm Bacteria of Osteomyelitis

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Biofilm bacteria complicate treatment of osteomyelitis by providing resistant to antibiotics, requiring high doses of 10 to 100 times of standard antibiotic treatment. This high leads several adverse effects and toxicity. To overcome these challenges, Gentamicin and \textit{Nigella sativa}, a natural product with antimicrobial property, are fused in an emulsion. Four emulsions (A,B,C, and D) (containing 0.1% w/v Gentamicin and \textit{N.sativa} oil concentration between 32.5% v/v to 46.6% v/v) were tested against common osteomyelitic, gentamicin-resistant, biofilm bacteria namely \textit{S.aureus}, \textit{S.epidermidis} and \textit{P.aeruginosa}. Test bacteria were isolated by sonicating clinical samples (Hospital Tengku Ampuan Afzan, Kuantan) and identified using colony morphology, biochemical test and API identification system (Bioemeriuex). Each strain was then tested for biofilm producing ability, followed by sensitivity testing to gentamicin. Antimicrobial susceptibility testing was performed using Disc Diffusion Assay by loading 20\textmu l of test product in blank disc placed on a plate that had been spread with test bacteria. Zone of inhibition was measured after 24 hours incubation. Finally, biofilm formation inhibition was evaluated by transferring emulsions (20\textmu l), test bacteria (100\textmu l) and broth (80\textmu l) per well of a 96-well culture plate and incubated for 24 hours at 37°C. After incubation, wells were decanted, washed 3 times with phosphate buffer solution, stained with 1% crystal violet before OD readings were collected using microplate reader. Results are analyzed using one way ANOVA. Emulsions were compared against \textit{N. sativa} alone, Gentamicin alone, and untreated sample as controls. The result of disc diffusion, indicated by diameter of zone of inhibition, revealed that significant different are seen against Gentamicin (Tukey’s, \( p \) value < 0.05, all emulsions) and \textit{N. sativa} alone (Tukey’s, \( p \) value < 0.05, emulsions A and C) against \textit{S.aureus} and \textit{S.epidermidis}. In inhibiting biofilm formation, both emulsions and \textit{N. sativa} alone have significant activity in comparison to Gentamicin (Tukey’s, \( p \) value < 0.05). No activity was exhibited against \textit{P.aeruginosa}. In conclusion, significantly improved anti-microbial and anti biofilm effects are seen against \textit{S.aureus} and \textit{S.epidermidis} but not \textit{P.aeruginosa}, hence the emulsions have a good prospect to be used future as an effective treatment against some gentamicin-resistant, biofilm bacteria.
Background: Epigenetics state of chromatin controls promoter activity, favoring either activation or silencing. A number of epigenetic regulators, such as DNA methyltransferase 1 (DNMT1) and G9a, a histone methyltransferase, have been implicated in various diseases including HIV-1 latency. In addition, the Ubiquitin-like, containing PHD and RING finger domains 1 (UHRF1) that is known to bind G9a has recently been recognized as a key epigenetic effector in mammalian cell transcription. In this study, we investigated the role of UHRF1 in HIV latency.

Methods: siRNA molecules were transduced into recipient cells and the effects of gene knock down was evaluated by transient luciferase assay. We performed immunoprecipitation/Western blotting using TZM-bl cell lysate to detect the binding of endogenous UHRF1 protein with transcription repressors such as G9a and DNMT1. Chromatin immunoprecipitation was used to determine the occupancy of UHRF1 and other transcriptional repressors at the HIV-1 LTR as well as the methylation state of histones.

Results: We found that UHRF1 negatively regulates HIV-1 LTR promoter activity. In fact, siRNA-mediated UHRF1 knock down induced HIV transcription. Thus, we further examined if UHRF1 would be coimmunoprecipitated with G9a and DNMT1, the downmodulators of HIV-1 transcription. Interestingly, we found that UHRF1 is associated with G9a and DNMT1 in non-stimulated TZM-bl cells, a cell model for HIV-1 latency containing a chromatin-integrated HIV-1 LTR. UHRF1 was dissociated from G9a and DNMT1 upon TNF-mediated stimulation of HIV transcription. In addition, UHRF1 protein was endogenously expressed in HIV-1 latently infected ACH2 and OM10.1 cells and was found to occupy the HIV-1 LTR promoter together with increased levels of histone methylation.

Conclusion: UHRF1 regulates HIV latency via recruiting G9a and DNMT1 and likely to be modified upon transcriptional induction such as by TNF-signaling. However, the function of UHRF1 in HIV latency is still unclear. Thus, it is necessary to further investigate the involvement of UHRF1 as it may be a key target for anti-HIV therapy.
Clinical features and risk factors for multidrug-resistant pathogens in community-acquired and healthcare-associated pneumonia in a Japanese community hospital

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Background: Identification of the causative pathogens is important for treatment of pneumonia. To manage patients with pneumonia who are found to be at risk of having multidrug-resistant (MDR) pathogens with high mortality and seem to be treated with broad-spectrum antimicrobials, healthcare-associated pneumonia (HCAP) was defined according to the 2005 American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) guidelines. However, broad-spectrum antibiotic therapy is not necessary in all HCAP patients, because the frequency of MDR pathogens varies according to the geographic region and medical environment. The objective of this study was to evaluate clinical characteristics and risk factors for MDR pathogens among patients with community-acquired pneumonia (CAP) and HCAP, both of which occur in communities.

Methods: We conducted a retrospective study of 219 adult patients hospitalized due to CAP (151 patients) and HCAP (68 patients). Methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas species, Acinetobacter species, and extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae were defined as MDR pathogens. Clinical features were compared and risk factors for MDR pathogens were analyzed among patients with CAP and HCAP.

Results: MDR pathogens were more frequently found in patients with HCAP (16.2%) than in patients with CAP (4.6%) (p=0.007). Multivariate logistic regression analyses for risk factors of MDR pathogens were presented in Table. These factors were independently correlated with isolation of MDR pathogens. Further, thirty-day mortality was higher in HCAP (13.2%) than in CAP (2.6%) (p=0.004). However, only one patient of 18 patients with MDR pathogens (5.6%) died within 30 days and this mortality was similar to that of patients without MDR pathogens (6.0%) in all CAP and HCAP patients. Ten of 12 patients with MRSA isolation seemed to be colonized, because they recovered without administration of anti-MRSA drugs.

Conclusion: Several host conditions were useful for predicting isolation of MDR pathogens. It is important to differentiate between infection and colonization for appropriate antibiotic therapy in CAP and HCAP patients.

Table. Multivariate analyses of risk factors for MDR pathogen in CAP and HCAP patients

<table>
<thead>
<tr>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
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<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric acid-suppressants</td>
<td>13.950</td>
<td>1.343-144.931</td>
<td>0.027</td>
</tr>
<tr>
<td>Aspiration</td>
<td>35.108</td>
<td>2.952-417.591</td>
<td>0.005</td>
</tr>
<tr>
<td>immunosuppression</td>
<td>35.264</td>
<td>2.890-430.236</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>HCAP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspiration</td>
<td>20.449</td>
<td>1.645-254.217</td>
<td>0.019</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>18.827</td>
<td>1.834-193.226</td>
<td>0.013</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td>247.859</td>
<td>4.889-12564.751</td>
<td>0.006</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>0.330</td>
<td>0.123-0.886</td>
<td>0.028</td>
</tr>
<tr>
<td>CAP severity measurement index</td>
<td>4.849</td>
<td>1.322-17.788</td>
<td>0.017</td>
</tr>
<tr>
<td><strong>All patients</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Aspiration</td>
<td>14.870</td>
<td>3.709-59.613</td>
<td>&lt;0.001</td>
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<tr>
<td>Chronic pulmonary disease</td>
<td>5.235</td>
<td>1.438-19.066</td>
<td>0.012</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td>14.928</td>
<td>2.428-91.783</td>
<td>0.004</td>
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<tr>
<td>Immunosuppression</td>
<td>18.277</td>
<td>3.996-83.605</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Outbreak of KPC-17 carbapenem-resistant *Klebsiella pneumoniae* in a regional hospital of southern Taiwan

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**Background:**
Carbapenem-resistant *Enterobacteriaceae* (CRE) infection is usually associated with inadequate empirical treatment and higher mortality. The prevalence rate of CRE in Taiwan is relatively low. We described a *Klebsiella pneumoniae* carbapenemase (KPC)-producing carbapenem-resistant *K. pneumoniae* (CRKP) outbreak since April 2014 in a regional hospital of southern Taiwan. Infection control strategies were introduced and the trend of transmission decreased gradually.

**Methods:**
Epidemiologic investigation of the outbreak was performed. Aggressive interventions such as active surveillance, strict contact isolation, cohort of patient and staff, were implemented to prevent further transmission. Trends of CRKP prevalence were evaluated and pulse-field gel electrophoresis (PFGE) was performed to assess the genetic relatedness of the isolates.

**Results**
We investigated 32 cases of KPC-producing CRKP, which were identified in a period of 4 months. Most of the patients came from nursing home, who had been hospitalized and received antibiotics therapy previously, and had chronic kidney disease. An outbreak at an ordinary ward was noted, which was then followed by an outbreak within an intensive care unit (ICU). Most of the isolates were cultured from urine or sputum and two of the three patients who had CRKP bacteremia expired due to septic shock with multi-organ failure. All tested isolates were confirmed KPC-17-producing *K. pneumoniae*, and all isolates were genetically related. Incidence of new CRKP cases decreased gradually after 4 months of management. Nevertheless, we still found some patients from nursing home carrying KPC-producing CRKP while performing active surveillance.

**Conclusion:**
The transmission of CRE can be reduced through aggressive infection control intervention. However, we believed that there was already an outbreak of KPC-producing CRKP within healthcare facilities in southern Taiwan. It will be a major challenge in the future especially during managing infections of the patients from the nursing home. Further public health intervention may be required in the era of multidrug resistant.
An Increasing Threat in the Intensive Care Unit: Multidrug-resistant *Acinetobacter baumannii*

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**Background**

Multidrug-resistant (MDR) *Acinetobacter baumannii* has emerged as a leading opportunistic pathogen in the ICU. These infections are extremely difficult to treat because of resistance to commonly prescribed antibiotics. This study aims to determine the genetic relatedness and antibiotic sensitivities of these strains and clinical features of these patients.

**Methods**

The study involved patients admitted to the adult ICU of a tertiary hospital in Malaysia (December 2011 to June 2012). All patients from whom *A. baumannii* was isolated from blood or bronchoscopic aspirates (BA) were included. Molecular characterization was carried out by PFGE, E-test was performed to determine MICs for various antibiotics and clinical data was extracted from medical notes.

**Results**

During the 7-months study period, 1023 patients were admitted to the ICU and 44 *A. baumannii* strains (blood, n= 20 and BA, n=24) were recovered. Six strains were randomly selected from non-ICU patients. All bronchoscopic aspirate strains were clinically regarded as colonizers. Recent antibiotics were administered to 95% patients; commonest were broad-spectrum penicilllin (62.5%), broad-spectrum cephalosporins (42.5%) and carbapenems (45%). All patients were mechanically ventilated and had central venous catheters. Mean (±SD) for the following were: length of ICU stay 16(10.9) days, APACHE II scores 23(6.85), number of recent invasive devices/procedures 3.38(0.63). Polymyxin B was the main antibiotic used to treat bacteraemic patients followed by sulperazone. Resistance rates were: cefoperazone/sulbactam (58%), ampicillin/sulbactam (76%), meropenem (84%), doripenem (84%) and amikacin (68%). All isolates were sensitive to polymyxin B. Twenty-six PFGE profiles were generated from *Apa*I-digested chromosomal DNA of 50 *A. baumannii* with DNA fragments ranging from 17 to 29 with sizes from 25.9 kb to 680.1 kb. Cluster analysis of the PFGE profiles based on 85% similarity generated two main clusters. An endemic clone was observed in which 20 strains isolated at different times, different ICU wards and from different patients had an indistinguishable PFGE profile. Multiple subtypes of closely related clones persisted in the ICU environment. Two of the 6 non-ICU strains had the same PFGE subtype as the members of the endemic clone, indicating the spread of the strains.

**Conclusion**

High evidence of multi-drug resistance and strain clonality was found amongst the *A. baumannii* strains in the ICU. Early identification of strain clonality and the application of infection control procedures with appropriate use of antibiotics are necessary to prevent the emergence and spread of MDR *Acinetobacter*. 
Direct detection of common Candida species in clinical blood samples by multiprobe real-time polymerase chain reaction
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Background: Candida species cause nosocomial blood stream infection with high mortality rate. Rapid detection and species identification are important for prompt antifungal therapy. We previously developed a rapid and specific single-tube multiprobereal-time polymerase chain reaction (RT-PCR) assay with multiple hybridization probes for detection of four common Candida species in blood culture broth. This study was an application of the assay to identify those species directly in patient blood samples.

Methods: Eighty-three blood samples were collected from 35 patients with candidemia and 48 patients without candidemia. DNA were extracted from 200 µl each of whole blood, buffy coat and plasma portions. DNAsamples were used as templates for multiprobe RT-PCR that contain four probes in a single tube. Amplification curves and melting peaks were analysed.

Results: By using multiprobe RT-PCR, 16 blood samples from candidemic patients were positive, which were detected in 9/16 C. tropicalis, 2/9 C. albicans, and 1/2 C. glabrata culture-positive blood samples. Mixed Candida species were detected in four blood samples by RT-PCR, which were 2 C. tropicalis/C. albicans, 1 C. parapsilosis/C. albicans and 1 C. parapsilosis/C. tropicalis blood samples. There were 3 culture-negative blood samples had positive RT-PCR results. Therefore, the assay yielded 93.75 % specificity and 45.71 % sensitivity with a positive predictive value of 84.21 % and a negative predictive value of 70.31 %. The most suitable blood portions for RT-PCR were buffy coat, which yielded positive results in 16 out of 19 samples (84.21 %). Whereas 10 out of 19 (52.63 %) were detected in whole blood, which was similar to the rate of detection in plasma (52.63 %). The lowest limit of detection in whole blood was 1-9 cells per reaction.

Conclusion: The single-tube multiprobe RT-PCR can be used for rapid direct detection of four common Candida species in blood samples within 3-hour turn-around time, which is useful for early antifungal treatment. However, a negative RT-PCR result does not rule out the possibility of candidemia. Buffy coat gave a highest yield for RT-PCR results.
Back to nature: herbal extracts antibacterial activity
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High Institute of Public Health, Alexandria University, Egypt

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.
Epidemiology of Short-term Peripheral Catheter-related Blood Stream Infection and Infection Control Efforts in a Japanese Tertiary Hospital

National Center for Global Health and Medicine, Tokyo, Japan

Background:
Catheter-related blood stream infection (BSI) is a major hospital-acquired infection worldwide. Disinfection using chlorhexidine gluconate (CHG) is effective for preventing central line-associated BSI. In Japan, peripherally inserted central venous catheters are not widely used; many patients receive drip infusion through short-term peripheral catheters (PC). The purpose of this study was to examine the epidemiology of PC-related BSI (PCRBSI) and the infection control needed to decrease the PCRBSI incidence.

Methods:
The National Center for Global Health and Medicine consists of 22 wards. We reviewed the PCRBSI incidence over 21 months beginning in April 2012. Of those reporting higher PCRBSI incidences, we selected 5 general wards and re-trained the staff regarding the appropriate procedure for insertion, maintenance, monitoring, and replacement of PC. Furthermore, antiseptic skin preparation was modified from 70% ethanol (ETOH) to a combination of ETOH and CHG. We began these interventions in January 2014 and observed the resulting PCRBSI incidence. Due to the limited availability of CHG formulations in Japan, an individually packaged 0.2% CHG formulation was used. The skin was disinfected with ETOH followed by CHG because the antiseptic effect of CHG lasts longer on human skin. The availability of CHG in the wards was regularly checked and its use encouraged. PCRBSI was defined based on the Infectious Diseases Society of America guidelines.

Results:
The hospital-wide PCRBSI incidence was 0.15/1000 patient-days during the pre-intervention period. After the interventions began, the mean PCRBSI incidence in the wards decreased from 0.23 to 0.09/1000-patient days (P for trend < 0.01). The most commonly isolated pathogens were Gram-positive cocci (37%) but Gram-negative pathogens were also identified (27%). The proportion of each pathogen did not differ between the pre and post intervention periods. No side effects associated with CHG use were observed.

Conclusion:
Disinfection with ETOH and CHG is a safe and feasible method to reduce PCRBSI incidence, especially when PC is frequently used. Further, longer studies are warranted to examine the cost and effective concentration of CHG and to evaluate the use of CHG.
Clinical Profile of Culture Positive Melioidosis Patients in Kapit Hospital, Sarawak.

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**Introduction:**
Melioidosis is a potentially fatal infection, caused by Burkholderiapseudomallei. It is a disease that is seen in Thailand, Malaysia and Northern Australia. Kapit district of Sarawak, is an endemic hot spot for melioidosis. However, data on the epidemiology, clinical manifestation, treatment and outcome of the disease in this part of the country are still very limited.

**Objective:**
1. To identify patient profile and clinical features of culture proven melioidosis.
2. To establish possible risk factors, treatment and outcome associated with melioidosis.

**Methodology:**
A cross sectional study of confirmed culture positive (including blood, sputum, urine and swab culture) Burkholderiapseudomallei cases in patients admitted to Kapit Hospital Sarawak from June 2012 to June 2014 will be undertaken. Information on culture positive Burkholderiapseudomallei will be retrieved from the Kapit Hospital laboratory record. The patients case notes will subsequently be traced and the relevant information will be entered into a pre-designed data collection form. Data will be analysed and interpreted via IBM SPSS Statistics version 20.0.

**Results:**
There were a total of 34 patients with the male to female ratio of 1:1. Majority of them were of Iban ethnicity (88.2%) The mean age for patient was 30 years old (range 1 year to 74 years old). Among the adult patients (n=18, 52.9%), 10 of them (56%) were involved in farming or forestry industry. Majority of the patients did not have medical co-morbidities (n=26, 76.5%). Interestingly, in terms of clinical manifestation, paediatric patients (n= 10 ) has a higher proportion of soft tissue abscesses as compare to adult patients (n= 16) who has a higher proportion of bacteremic cases ( p < 0.05). Majority of the cultures were sensitive to Gentamycin (91.1%), Ceftazidime (97.1%), Imipenem (100%) and Meropenem (100%). Most patient received ceftazidime (76%) followed by meropenem (15%) and imipenem (9%). Mortality rate was 24% (n=4) More bacteremic patients (50%, n=10) admitted to ICU compared to non bacteremic patients. Higher proportion of death is noticed in younger patient (<60 years old). Patients who suffered from respiratory failure, renal failure, metabolic acidosis, septic shock and requiring ICU care, ventilation or inotropic support also has a higher proportion of death (p<0.05).

**Conclusion:**
There were a few obvious differences when it comes to clinical profile of melioidosis patients in Kapit area compared to our counterparts in Thailand and Australia. The patients were younger and without risk factors. Burkholderiapseudomallei in Kapit region is sensitive to gentamycin, and the clinical presentation in paediatric (soft tissue abscesses) and adult (bacteremic) group were different. High suspicion and familiarity with the pattern of the disease course unique to Kapit region is crucial in ensuring early treatment and preventing mortality.

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Objectives: Streptococcus pneumoniae (SP) and Haemophilus influenzae (HI) are important community-acquired respiratory tract pathogens and antibacterial resistance among these is a problem in many parts of the world and especially in Asia. SP and HI data from the SOAR programme in Singapore are reported.

Methods: In 2013 and 2014, 58 SP and 50 HI were collected from Singapore. MICs for various antimicrobials were determined using Etest or disk susceptibility testing (according to CLSI guidelines). Susceptibility was assessed using CLSI interpretive criteria except for azithromycin and clarithromycin where bioMerieux Etest breakpoints in CO₂ were used.

Results: Overall, 67.2% (39/58) of SP were penicillin-susceptible and 32.8% (19/58) were penicillin-non-susceptible based on CLSI oral penicillin breakpoints. Using CLSI IV penicillin breakpoints, 96.6% (56/58) were susceptible to penicillin. Overall, susceptibility to moxifloxacin, levofloxacin, amoxicillin/clavulanic acid, cefuroxime, cefaclor, clarithromycin, erythromycin, trimethoprim-sulfamethoxazole and ofloxacin was 100% (58/58), 98.3% (57/58), 96.6% (56/58), 82.8% (48/58), 70.7% (41/58), 60.3% (35/58), 58.6% (34/58), 44.8% (26/58) and 43.1% (25/58), respectively. For HI, 18.0% (9/50) produced beta-lactamase and 1 isolate (0.5%) was beta-lactamase-negative but ampicillin resistant (BLNAR). All HI were fully susceptible to azithromycin, ciprofloxacin, levofloxacin and ofloxacin. Susceptibility was also >90% to moxifloxacin and amoxicillin/clavulanic acid at 98.0% (49/50) and 94.0% (47/50), respectively. Cefuroxime, clarithromycin, cefaclor and trimethoprim-sulfamethoxazole were less active with susceptibility of 88.0% (44/50), 84.0% (42/50), 78.0% (39/50) and 58.0% (29/50), respectively.

Conclusions: Among SP strains, moxifloxacin, levofloxacin, amoxicillin/clavulanic acid were the most active antibacterials but penicillin non-susceptibility was high. Low susceptibility was also observed for macrolides, trimethoprim-sulfamethoxazole and ofloxacin. For HI strains, azithromycin, fluoroquinolones and amoxicillin/clavulanic acid retained very good in vitro activity but beta-lactamases were detected in 18.0% of isolates. Other agents, especially trimethoprim-sulfamethoxazole, were also poorly active. Antibacterial resistance was found to be relatively high in Singapore, so continued surveillance
is necessary to track further changes in susceptibility over time.
Synergistic effect of hibicuslide C against antibiotic-resistant *Pseudomonas aeruginosa*

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Background: *Pseudomonas aeruginosa* is naturally resistant to many antimicrobial agents, and infections induced by this strain are serious diseases, especially to hospitalized patients. The countermeasure of these infection was indicated that synergistic effect between phytochemical and clinical antibiotics. The objective of this study is to determine the interaction between hibicuslide C derived from *Abutilion theophrasti*, and standard antibiotics against antibiotic resistant *P. aeruginosa* (ARPA).

Methods: In this study, the minimum inhibitory concentration (MIC) of the compounds was compared with that of the standard antibiotics against ARPA obtained from patients directly using two dilution methods. Additionally, the interaction with norfloxacin was estimated by calculating the fractional inhibitory concentration index (FICI) for each combination to determine synergy effect.

Results: the results of the antibacterial assays indicated that the compounds exert antibacterial activities, with MIC values ranged from 5.0 to 10.0 µg/ml. Hibicuslide C possessed antibacterial effects and all combination showed synergistic effects against ARPA. Furthermore, these strains were found to interact synergistically in biofilms formed in 96-well microtiter plates.

Conclusion: hibicuslide C have shown antipseudomonal activities and although independently weaker than standard antimicrobial agents, synergistic effect of this compound was potent. Combinations of hibicuslide C with norfloxacin have potential as a therapeutic agent and adjuvant for treatment of bacterial infection.

**Key words**
Hibicuslide C; Synergistic effect; *Pseudomonas aeruginosa*
Role of regulatory T cells and Th17 cells in *Helicobacter pylori* gastritis

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**Background:** *Helicobacter pylori* persists for the virtual life of the host. Recent studies have suggested that CD4+CD25+FoxP3+ regulatory T cells may be involved in this process. The aim of this study was to study T cell immune response in relation to the density of *H. pylori* colonization and degree of gastric inflammation.

**Methods:** By using blood samples of 8 *H. pylori* infected patients and 44 non-infected dyspeptic patients as the control, we used flow cytometry to quantify the number of CD4+CD25+CD127dim cells and IL-10 and IL-17 levels were quantified by ELISA.

**Results:** There was a positive correlation between CD4+CD25+CD127dim counts and the degree of infiltration of mononuclear cells from absent, mild, moderate to marked infiltration in both *H. pylori* positive and negative patients. The IL-10 levels were higher in patients infected with *H. pylori*. Furthermore, it was seen that there was a negative correlation between IL-10 and mucosal mononuclear infiltration. The levels of IL-17 was not detected in plasma samples of patients.

**Conclusion:** Increased regulatory immune response results in *H. pylori* persistence and chronic inflammatory response.
Impact of Carbapenems Stewardship in Medical Wards in UKMMC

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P Periyasamy,
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Background
There were 4 carbapenems available and the consumption was increasing collectively each year. Among medical wards, carbapenems usage was noted to incline from 50.6 DDD per 1000 patient days in year 2008 to 90.3 DDD per 1000 patient days in year 2011. The increasing trend was worrying and showed that the routine offormulary restriction, pre-authorization approaches and antibiotic guideline already in place were insufficient. This indicated the need for further intervention to curb the issue.

Methods
Since Aug 2011, carbapenems stewardship was implemented adopting prospective audit and feedback approach. 6 clinical pharmacists in 6 medical wards identified patients who were prescribed carbapenems. One Infectious disease physician and medical officer were notified of case through phone short messages and they reviewed the cases to determine if the prescribing was rational. In addition, case discussions were held once a week by patient bedside. Suggestions were either documented in patient care notes or communicated personally. Clinical pharmacists continued to follow up cases to document the progress and acceptance of suggestion.

Results
In year 2013, total 150 cases were identified and reviewed. 40 suggestions were given which consisted of change of antibiotics, change dosage or duration or discontinuation. The acceptance rate in 2013 was 82.5% (33/40) which was consistent with rate in year 2012 (87.9%, 29/33).

Comparing to year 2011, the consumption of carbapenems had decreased by 25% in year 2013. The rate of carbapenems usage was decreasing progressively to 71.74 DDD per 1000 patient days in year 2012 and 67.84 patients days in year 2013. The same was observed in individual group 2 carbapenems (47.3% decrease in Imipenem, 31.1% decrease in Meropenem and 37.7% decrease in Doripenem), though an increase (67.0%) was observed in Ertapenem usage.

Conclusion
The implementation of stewardship programme was well received by medical discipline. This had contributed to a decrease in carbapenems usage and it was still in decreasing trend after 2 years of implementation. However, the current approach was labour intensive. Further study was required to examine the impact on patient outcomes.
The interface between MRSA vancomycin resistance and its virulence potential: a positive association in the Caenorhabditis elegans infection model

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Background:
Staphylococcus aureus is a medically important pathogen commonly associated with antimicrobial resistance and patient morbidity. However, it remains unclear whether antimicrobial resistance is associated with S. aureus virulence and its effect on host infection outcome. Vancomycin, a glycopeptide, is currently used as the first line antibiotic for methicillin-resistant S. aureus infections. In a previous study, chromosomal engineering of Mu50Ω¹, a vancomycin-susceptible S. aureus (VSSA) (vancomycin minimum inhibitory concentration (MIC) = 1mg/L), generated strains Mu50Ω¹-vraSm and Mu50Ω¹-vraSm-graRm which are vancomycin-intermediate S. aureus (VISA) with vancomycin MICs of 4 and 6mg/L, respectively (Cui et al., Antimicrob Agents Chemother. 2009 Mar; 53(3):1231-4). Mu50Ω¹, Mu50Ω¹-vraSm and Mu50Ω¹-vraSm-graRm are isogenic strains, whereby Mu50Ω¹ and Mu50Ω¹-vraSm are completely identical in their whole genome sequence except for a vraSm mutation (generating a stop234 → methionine234 substitution) in Mu50Ω¹-vraSm, while the whole genome sequence of Mu50Ω¹-vraSm and Mu50Ω¹-vraSm-graRm differs only in a graRm mutation (generating an Asn197 → Ser197 substitution) in Mu50Ω¹-vraSm-graRm. The isogenic nature of these strains makes them ideal tools to investigate the impact of vancomycin resistance triggered by the vraSm and graRm mutations towards MRSA virulence.

Methods:
Using Caenorhabditis elegans strain N2 as an infection model, strains Mu50Ω¹, Mu50Ω¹-vraSm and Mu50Ω¹-vraSm-graRm were fed to the worms over a period of 14 days. The number of dead and surviving worms was scored for every tested strain each day during the assay; worms that crawl off the bacterial lawn were excluded from the scoring. Worm survival (%) for the experiment duration was then plotted against time (day) in a Kaplan-Meier plot.

Results:
From this study, we found that VISA strains Mu50Ω¹-vraSm and Mu50Ω¹-vraSm-graR killed the worms in a shorter duration (2 to 10 days) compared to the VSSA strain Mu50Ω¹. In addition, some worms (35.9%) fed with Mu50Ω¹ were still alive at the end of the assay duration. Interestingly, strain Mu50Ω¹-vraSm showed the highest virulence potential amongst the 3 tested strains; the killing was complete in just 2 days.

Conclusion:
This study shows that besides mediating vancomycin resistance, the vraSm mutation appears to be also important for S. aureus virulence and infection; while the graRm mutation enhances vancomycin resistance but attenuates virulence potential of the bacteria. Further investigation into the importance of these 2 mutations will enable a deeper understanding of S. aureus vancomycin resistance and its virulence potential.
Clinical response by study district in the Asian community-acquired pneumonia (CAP) trial of ceftaroline fosamil 600mg every 12 hours vs ceftriaxone 2g every 24 hours

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Background: A recent Phase III, randomised, double-blind trial (NCT01371838) demonstrated that ceftaroline fosamil 600 mg every 12 hours (q12h) was superior to ceftriaxone 2g per day (q24h) in Asian patients hospitalised with Pneumonia Outcomes Research Team (PORT) risk class 3–4CAP (Zhong et al.ECCMID 2014). This analysis examines clinical response rates by study district (China, India, Korea, Taiwan, Vietnam).

Methods: Adults with PORT 3–4CAP requiring hospitalisation were randomised 1:1 to ceftaroline fosamil 600 mg q12h (n=385) or ceftriaxone 2 g q24h (n=386) for 5–7 days. The primary endpoint was the difference in clinical cure rates at the test-of-cure (TOC) visit (8–15 days after last dose) in the clinically evaluable (CE) population; non-inferiority was defined as a lower limit of the 95% CI ≥–10% for the between-group difference; superiority was defined as a lower limit of the 95% CI >0%. Differences in clinical cure rates by district were compared with the overall primary endpoint.

Results: Clinical cure rates at the TOC visit in the CE population overall (primary endpoint) and by study district are shown in the Table. Similar results were observed in the modified intent-to-treat population.

<table>
<thead>
<tr>
<th>District</th>
<th>Ceftaroline fosamil 600 mg q12h</th>
<th>Ceftriaxone 2 g q24h</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>217/258 (84.1)</td>
<td>178/240 (74.2)</td>
<td>9.9 (2.8, 17.1)</td>
</tr>
<tr>
<td>China</td>
<td>89/116 (76.7)</td>
<td>69/109 (63.3)</td>
<td>13.4 (1.4, 25.2)</td>
</tr>
<tr>
<td>India</td>
<td>58/61 (95.1)</td>
<td>51/55 (92.7)</td>
<td>2.4 (–7.4, 13.1)</td>
</tr>
<tr>
<td>Korea</td>
<td>38/45 (84.4)</td>
<td>29/40 (72.5)</td>
<td>11.9 (–5.7, 29.8)</td>
</tr>
<tr>
<td>Taiwan</td>
<td>20/21 (95.2)</td>
<td>12/18 (66.7)</td>
<td>28.6 (4.7, 52.9)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>12/15 (80.0)</td>
<td>17/18 (94.4)</td>
<td>–14.4 (–41.2, 9.9)</td>
</tr>
</tbody>
</table>

Conclusion: Clinical response rates in the larger districts were consistent with the primary analysis and generally favoured ceftaroline fosamil > ceftriaxone. Although the raw difference in Taiwan and Vietnam was inconsistent with the overall results, the patient numbers were very low (as reflected by the wide CIs), and thus the results are still in line with the larger districts and the primary analysis.
Bacterial pathogens causing postoperative infections following abdominal surgery at a tertiary care hospital in Southern Province of Sri Lanka

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Background

Hospital acquired infections are important causes of morbidity and mortality. Patients who undergo abdominal surgery are more prone for infections due to the invasive procedure and devices inserted for peri-operative care and monitoring. The general objective of the study was to study postoperative infections among patients undergoing abdominal surgery. Specific objectives were to find out the incidence of postoperative infections following abdominal surgery, to identify the common bacterial pathogens causing postoperative infections and their antibiotic sensitivity patterns and to determine the most effective empirical antibiotic/s for the treatment.

Methods

A prospective study was carried out at 5 general surgical units from January to April 2012. Patients were followed up for 30 days postoperatively to detect postoperative infections.

Results

Total number of the study population was 385. Thirty patients (7.8%) developed postoperative infections and total episodes of post-operative infections were 37(9.6%).

The commonest postoperative infection was surgical site infections (SSIs) with an incidence of 5.97% followed by urinary tract infections (UTI)1.82%, hospital acquired pneumonia/ventilator associated pneumonia (HAP/VAP)1.56% and catheter related blood stream infections (CRBSI) 0.26%.

Majority of SSIs (82.6%) were seen following laparotomy and 87% of these were superficial incisional SSIs.

Coliforms were the commonest pathogens isolated in SSIs (36.36%). Equal numbers of Staphylococcus aureus and Acinetobacter isolates were seen (18.18% each). Among coliforms 62.5% were ESBL producers. Acinetobacter isolates were resistant to almost all antibiotics. Fifty percent of S. aureus were MRSA.

Among seven pathogens causing UTI/CAUTI were 2 coliforms, 1 Acinetobacter species, 1 Enterococcus species and 3 Candida species. Both coliform isolates were ESBL producers.

Of the 5 pathogens isolated in HAP, there were coliforms (3/5), Pseudomonas species (1/5) and Streptococcus pneumoniae(1/5).

Acinetobacter species were isolated in both patients with VAP and were resistant to all tested antibiotics.

Pseudomonas aeruginosa isolated in CRBSI was resistant to carbapenems.

Conclusions
Surgical site infections were the commonest postoperative infections. UTI, HAP, VAP and CRBSI were also detected. Gram negative organisms were the predominant pathogens.

Gram negative organisms were the commonest pathogens causing SSIs. Majority of SSIs were superficial incisional SSIs in which wound cleaning alone may play an important role in the management. If antibiotics are indicated carbapenems and amikacin are the most effective empirical treatment.

ESBL production among coliforms (61.5% of all coliforms) is a significant problem when treating infections in this population. Carbapenem resistance was not detected among coliforms. But 81.8% of non-fermenters including Acinetobacter species and Pseudomonas species were resistant to carbapenems.
Soluble ST2 has a prognostic role in critically ill patients with suspected sepsis

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Running title: sST2 in sepsis

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Abstract

**Background:** Soluble suppression of tumorigenicity 2 (sST2) has emerged as a novel biomarker for heart failure and can be also increased in inflammatory diseases. We explored whether sST2 is related to cardiac dysfunction/failure and has a prognostic role in critically ill patients with suspected sepsis.

**Methods:** In a total of 397 patients with suspected sepsis, sST2 concentrations were measured using Presage ST2 Assay (Critical Diagnostics, CA, USA). sST2 concentrations were analyzed according to procalcitonin (PCT) concentrations, cardiovascular subscores of sepsis-related organ failure assessment (SOFA) score, and clinical outcomes.

**Results:** sST2 concentrations were significantly different according to the five groups of PCT concentrations and cardiovascular subscores of SOFA score ($P < 0.000001$ and $P = 0.036$, respectively). In-hospital mortality was significantly different between the two groups of sST2 concentrations at cut-off of 35 ng/mL ($P = 0.0213$). In the four groups according to the sST2 and PCT concentrations, the highest in-hospital mortality was observed when both sST2 and PCT concentrations were increased ($P = 0.0028$).

**Conclusion:** sST2 seems to be related to both cardiac dysfunction/failure and severity in sepsis. Combined use of sST2 and PCT would be promising for the risk stratification and prognosis prediction in critically ill patients with suspected sepsis.

**Keywords:** sST2, cardiac failure, severity, prognosis, procalcitonin, sepsis
Epidemiology of Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus* (VRE) and Carbapenem-resistant *Enterobacteriaceae* (CRE) in an acute care hospital and its affiliated intermediate- and long-term care facilities (ILTCs)

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**Background**

Antimicrobial resistance is a growing clinical and public health problem worldwide. Little is known about the prevalence and epidemiology of multi-drug resistant organisms (MDROs) in ILTCs. Our study aims to compare the prevalence of and risk factors for MDROs in an acute hospital with its affiliated ILTCs.

**Methods**

We conducted a period prevalence survey at an acute tertiary-care hospital in Singapore and in 5 of its affiliated ILTCs, from 02-June to 25-June 2014. Randomly selected patients with >48 hours stay in the acute hospital and all patients/residents from the ILTCs were included. Nasal, axilla & groin swabs were cultured for MRSA whilst rectal swabs/stool samples screened for VRE and CRE. Demographic and hospitalization data were extracted from administrative databases.

**Results**

A total of 1044 patients were screened: acute hospital (32.7%), intermediate-care facilities (ICFs) (34.5%) and long-term care facilities (LTCFs) (32.9%) respectively. MRSA prevalence in (ICFs) (30.0%) was the highest followed by (LTCFs) (20.4%) and the acute hospital (15.5%). The acute hospital had the highest VRE (18.9%) and CRE (3.0%) prevalence. The median ages, years (interquartile range, IQR) of the participants were 73 (62-82) for acute hospital, 72 (61-80) for ICFs, and 66 (58-77) for LTCFs. The median length of stay (LOS), days (IQR) at LTCFs was 1338 (480-2050), and 12 (6-27) and 21 (12-39) for acute hospital and ICFs. On multivariate analysis, male gender (adjusted OR (AOR) 1.61, 95% CI 1.18-2.21), LOS >14 days (AOR 1.70, 95% CI 1.14-2.53), residents of ICF (AOR 2.26, 95% CI 1.50-3.40) and VRE co-colonization (AOR 1.90, 95% CI 1.09-3.30) were independent risk factors for MRSA colonization after adjusting for age. MRSA co-colonization (AOR 1.78, 95% CI 1.02-3.10) and age >65 years (AOR 2.31, 95% CI 1.26-4.24) were positively associated with VRE colonization, after adjusting for gender, institution, LOS, and CRE co-colonization. CRE co-colonization (AOR 2.99, 95% CI 0.93-9.68) was marginally associated with VRE colonization.

**Conclusion**

MRSA prevalence in ILTCs was higher than in the acute hospital, although the reverse was observed for VRE and CRE. VRE colonization was associated with MRSA and CRE colonization respectively. Appropriate infection prevention and control strategies targeting patients colonized with VRE could be instituted, to reduce the prevalence and transmission of MDROs across the healthcare settings.
High incidence of penicillin resistant CoNS in a student community with limited presence of MDR and methicillin resistant isolates carrying a major community-associated SCCmec type

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Background: Coagulase negative staphylococci (CoNS), predominantly Staphylococcus epidermidis, are normal commensals of the skin and anterior nares. The issues on emergence of methicillin resistance (MR) and community acquired (CA) infection involving CoNS have recently gained concern. This study was conducted to evaluate the status of CoNS in relation to their nasal carriage, risk factors, antimicrobial resistance and genotype in a student population.

Methods: This study was conducted at Universiti Putra Malaysia in November 2013 involving 176 health science students. A self-administered questionnaire on socio-demographic and risk factors was distributed, followed by collection of nasal swab and cultivation to preliminarily differentiate CoNS from Staphylococcus aureus. Antibiotic susceptibility was determined by disc diffusion method according to CLSI’s guidelines. Only isolates that were resistant to at least one antibiotic were subjected to species identification based on tuf gene sequencing followed by meca gene screening and SCCmec typing using PCR.

Results: All participants were healthy and had not been hospitalized in the past 6 months. From 176 students, a total of 109 single isolates of CoNS (61.9%) was obtained. Chi-square and Fisher’s exact test showed significant association of nasal carriage of CoNS with the habit of touching nose (P=0.003). The other risk factors (gender, ethnicity, skin infection, health status and use of intravascular devices) were deemed as not significant. Resistance to penicillin was the highest among the isolates (62.4%) followed by tetracycline (9.2%), erythromycin (8.3%), cefoxitin (5.5%), ceftazidime (4.6%), gentamicin (2.8%) and rifampin (1.8%). Among 77 isolates that were subjected to tuf gene sequencing, 74 were identified as S. epidermidis and 3 as S. haemolyticus. Four isolates (S. epidermidis) were identified as multidrug resistant strains (MDR) for being resistant to more than 3 antimicrobial categories. meca gene was detected in 12 isolates (10 S. epidermidis; 2 S. haemolyticus); however, 6 of them were susceptible to methicillin (cefoxitin). Nine of meca positive isolates carried SCCmec type IV (8 S. epidermidis; 1 S. haemolyticus), 2 carried SCCmec type II (1 S. epidermidis; 1 S. haemolyticus) and 1 carried SCCmec type I (S. epidermidis).

Conclusion: In this study, majority of meca positive isolates carried SCCmec type IV, similar to that of the major CA-MRSA clones worldwide. The presence of MR-CoNS warrants further investigation on potential dissemination of MR-CoNS at a larger extent in the Malaysian community.
Droplet precautions are adequate for protection of healthcare workers managing mechanically ventilated patients with severe influenza
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National University Health System, Singapore

Abstract
Background
Influenza is one of the principle public health threats worldwide. The disease is highly contagious with and is characterized by seasonal epidemics and periodic pandemics upon introduction of new genotypes of the virus. Despite the enormous burden of the disease, there are surprisingly little data on the transmission of influenza virus to healthcare workers (HCWs) in the intensive care unit (ICU). There have been documented infections of healthcare workers (HCWs) in ICUs in various settings. In the present study, we report on the transmission of influenza in patients admitted to ICU to the healthcare workers using droplet precautions.

Methods
Upon laboratory confirmation of an influenza infection in an ICU patient, attending HCWs were invited to participate in the study. Consenting HCWs provided samples for serology by haemagglutination inhibition assay as well as nasal swabs and hand swabs for influenza polymerase chain reaction (PCR) by previously described methods. Body temperatures of HCWs were taken twice daily and they were instructed to complete diary cards recording contact time, PPE use and any symptoms. Four surfaces in ICU were also swabbed for virus shedding.

Results
73 HCWs attending 4 ventilated patients using droplet precautions were recruited. No virus was detected by PCR in the nasal or hand swabs of the HCW recruited into the study and none of volunteers were found to have seroconverted.

Conclusion
The data from this study indicate that there is little to no risk of transmission of Influenza from an infected patient to the healthcare workers or cleaners in the ICU with the use of routine droplet precautions in mechanically ventilated patients.
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 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 clinical specimens and culture isolates were sequenced. A total of 627 sequencing results between March 2011 and June 2014 from 67 hospitals nationwide were analyzed.

Results:
One mutation in the rpoB, katG and inhA genes were detected in 17.4% of specimens (109 of 627). The point mutations in the hot-spot of rpoB gene were detected in 67 (10.7%) specimens, which were found most frequently at codons 531 (59.7%), 516 (14.9%), 526 (10.4%), 511 (6.0%), 533 (4.5%) and 513 (4.5%) (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 67 specimens with mutations in rpoB gene, 30 (44.8%) had concurrent mutation in katG gene and 9 (13.4%) had concurrent mutation in inhA gene. Fifty-four (8.6%) specimens had mutations in the codon 315 of the katG gene. The katG gene was shown to bear mutations of two different types: S315T (98.1%), S315N (1.9%). The mutations in the inhA regulatory region were found in 26 (4.1%) of specimens. In the inhA gene, nucleotide substitutions at position -15C>T were most common mutation (80.8%), followed by -8T>C substitution (11.5%).

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
Membrane disruptive mechanism of the antimicrobial peptide Bac8c

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Background: Bac8c (RIWVIWRR-NH2) is an analogue peptide derived through complete substitution analysis of the linear bovine host defense peptide variant Bac2A. In the present study, the antifungal mechanism of Bac8c against pathogenic fungi was investigated, with a particular focus on the effects of Bac8c on the cytoplasmic membrane.

Methods: We used bis-(1,3-dibutylbarbituric acid) trimethineoxonol [DiBAC4(3)] staining to show that Bac8c induced disturbances in the membrane potential of Candida albicans. An increase in membrane permeability was also observed by the influx of propidium iodide in Bac8c-treated C. albicans. Furthermore, we studied the effects of Bac8c treatment on model membranes to elucidate its antifungal mechanism. Using calcein and FITC-labeled dextran leakage assays from Bac8c-treated large unilamellar vesicles (LUVs) and giant unilamellar vesicles (GUVs)

Results: We found that Bac8c has a pore-forming action on fungal membranes, with an estimated pore radius of between 2.3 and 3.3 nm. A membrane-targeted mechanism of action was also supported by the observation of potassium release from the cytosol of Bac8c-treated C. albicans.

Conclusion: These results indicate that Bac8c is considered as a potential candidate to develop a novel antimicrobial agent because of its low-cost production characteristics and high antimicrobial activity via its ability to induce membrane perturbations in fungi.

Keywords
Bac8c; Antimicrobial peptide; Antifungal; Plasma membrane; Candida albicans
Efficacy of BETADINE® and other preparations with antiseptic properties against a biofilm formed by *Pseudomonas aeruginosa*, and a multispecies biofilm of *Candida albicans* and Methicillin-resistant *Staphylococcus aureus* (MRSA)

M.J. Hoekstra,1 S Westgate,2 S Mueller3


**Background:** Microorganism-forming biofilms are among the leading causes of persistent infections in chronic wounds. Production of wound exudate as a result of the presence of microorganisms can be elevated significantly, thereby causing dilution of topical antiseptics. Consequently, agents that demonstrate a significant reduction in activity when diluted should be considered with caution for treatment of exuding chronic wounds. We assessed biofilm removal capabilities of eight diluted topical wound agents and a silver-impregnated wound dressing against *Pseudomonas aeruginosa* biofilms, multispecies biofilms of *Candida albicans* and methicillin-resistant *Staphylococcus aureus* (MRSA), which had been established using an in vitro biofilm model.

**Methods:** Repeatable *P. aeruginosa*, mixed *C. albicans* and MRSA biofilms were established on polystyrene coupons for 48 hours using a CDC reactor. Coupons were removed from the reactor and treated for 4 or 24 hours with the topical treatments or control. Treatments were tested at label concentration and after 1:10 dilution; BETADINE® was additionally diluted 1:3 and 1:30. Following treatment, coupons were rinsed three times in phosphate buffered saline to remove planktonic isolates. Remaining viable biofilm material was recovered by sonication and quantified using colony counts. All samples were tested in triplicate.

**Results:** BETADINE® showed excellent efficacy (>5 Log reduction in recoverable viable biofilm material compared to untreated controls) against pre-formed *P. aeruginosa* biofilms at all dilutions and over both 4 and 24 hours. Similar results were produced for all other topical treatments except those containing mupirocin 2% and fusidic acid 2%, which showed no significant difference compared to control samples after 1:10 dilution. The silver wound dressing resulted in no significant difference in *P. aeruginosadiosbiofilm cultures compared to the control samples after 4 hours; <2 Log reduction compared to the positive control was reported after 24 hours’ treatment. Treatment of mixed *C. albicans*/MRSA biofilms with a 1:10 dilution of BETADINE® produced a 2 Log reduction in viable biofilm cultures at 4 hours and >5 Log reduction at 24 hours – representing the most effective action for any of the diluted agents. **Conclusion:** In addition to preventing the recovery of viable *P. aeruginosa* biofilm material, BETADINE® diluted up to 10-fold from the commercial preparation also prevented the recovery of viable organisms from multispecies biofilms of *C. albicans* and MRSA. The efficacy of diluted BETADINE® in this in vitro model indicates its potential effectiveness for the treatment of exuding chronic wounds containing multispecies biofilms.

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Comparative Ceftaroline (CPT) Activity against Skin and Soft Tissue Infection (SSTI) Pathogens from Asia-Pacific Region (APR) AWARE (Assessing Worldwide Antimicrobial Resistance Evaluation) Surveillance Program 2013

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Background: In APR, antimicrobial resistance is a serious public health concern. Surveillance programs provide physicians with current susceptibility profiles of major pathogens. This report summarizes the comparative activity of CPT, a new cephalosporin with activity against common SSTI pathogens including methicillin-resistant Staphylococcus aureus (MRSA) against SSTI pathogens collected across APR territories.

Methods: 2,153 SSTI isolates were collected from 35 medical centers located in Australia (5), China (11), Japan (4), South Korea (3), Malaysia (3), Philippines (3), Taiwan (3) and Thailand (3) in 2013. Broth microdilution susceptibility testing was performed and interpreted according to CLSI guidelines. Enterobacteriaceae were screened for extended-spectrum β-lactamases (ESBLs) using ceftazidime, aztreonam and cefotaxime MICs (<2 mg/L).

Results: Percent susceptibility (% S) of selected pathogens versus antimicrobials tested are presented in the table.

<table>
<thead>
<tr>
<th>Pathogen (n)</th>
<th>CPT</th>
<th>AMC</th>
<th>FEP</th>
<th>TZP</th>
<th>LVX</th>
<th>AZT</th>
<th>TGC</th>
<th>ERY</th>
<th>CLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus (1,365)</td>
<td>92.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>73.6</td>
<td>-</td>
<td>97.6</td>
<td>55.8</td>
<td>74.3</td>
</tr>
<tr>
<td>MRSA (757)</td>
<td>85.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>55.6</td>
<td>-</td>
<td>95.8</td>
<td>38.7</td>
<td>59.6</td>
</tr>
<tr>
<td>MSSA (608)</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>95.9</td>
<td>-</td>
<td>99.8</td>
<td>77.1</td>
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</tr>
<tr>
<td>S. pyogenes (183)</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>98.4</td>
<td>-</td>
<td>100</td>
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<tr>
<td>S. agalactiae (38)</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>86.8</td>
<td>-</td>
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<td>S. dysgalactiae (34)</td>
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<td>100</td>
<td>-</td>
<td>97.1</td>
<td>73.5</td>
<td>91.2</td>
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<td>E. coli (185)</td>
<td>57.8</td>
<td>60.5</td>
<td>76.8</td>
<td>92.4</td>
<td>55.1</td>
<td>72.4</td>
<td>99.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ESBL-negative(120)</td>
<td>88.3</td>
<td>77.5</td>
<td>100</td>
<td>94.2</td>
<td>74.2</td>
<td>100</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>K. pneumoniae (100)</td>
<td>58</td>
<td>61</td>
<td>78</td>
<td>80</td>
<td>91</td>
<td>71</td>
<td>97</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ESBL-negative(63)</td>
<td>92.1</td>
<td>90.5</td>
<td>100</td>
<td>95.2</td>
<td>95.2</td>
<td>100</td>
<td>98.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K. oxytoca (14)</td>
<td>85.7</td>
<td>78.6</td>
<td>92.9</td>
<td>85.7</td>
<td>100</td>
<td>85.7</td>
<td>92.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ESBL-negative(12)</td>
<td>100</td>
<td>91.7</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteus mirabilis (71)</td>
<td>87.3</td>
<td>87.3</td>
<td>98.6</td>
<td>98.6</td>
<td>83.1</td>
<td>100</td>
<td>45.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ESBL-negative(67)</td>
<td>92.5</td>
<td>92.5</td>
<td>100</td>
<td>100</td>
<td>85.1</td>
<td>100</td>
<td>44.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. morganii (24)</td>
<td>79.2</td>
<td>4.2</td>
<td>95.8</td>
<td>95.8</td>
<td>83.3</td>
<td>95.8</td>
<td>91.7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

AMC = amoxicillin-clavulanate, CRO = ceftriaxone, FEP = cefepime, CPT = ceftaroline, TZP = piperacillin-tazobactam, LVX = levofloxacin, AZT = aztreonam, TGC = tigecycline, ERY = erythromycin, CLI = clindamycin, “-” (not indicated)

Conclusions: S. aureus (SA) was a common cause of SSTIs in APR. CPT was highly active against SA (92% susceptible) while Enterobacteriaceae were less frequently encountered. 70.8% of isolates collected screened negative for ESBL production. Similar to other β-lactams, CPT % susceptibility was impaired by the production of serine β-lactamases.
Title: “Clinical Score to differentiate Scrub typhus and Dengue (CSSD)” - an useful tool to differentiate between scrub typhus and dengue febrile illness, the two emerging diseases.

Authors:
2. Abhilash KPP, Associate Professor, Christian Medical College, Vellore.
5. John A Jude, Professor of Microbiology, Christian Medical College, Vellore.

Background: Dengue and scrub typhus (ST) shares similar clinico-epidemiological features and is difficult to differentiate at acute presentation. While the mainstay of treatment for dengue is supportive, but the treatment of ST requires doxycycline. We aimed to develop a scoring model, “Clinical Score to differentiate Scrub typhus and Dengue (CSSD)”, which can differentiate between scrub typhus and dengue febrile illness.

Methods: In this cross-sectional study at tertiary care hospital in south India, 370 adult (>16 years) patients with acute (<14 days duration) febrile illness were enrolled from September 2012 to April, 2013. Case of scrub typhus and dengue febrile illness was classified as per predefined standard criteria. Univariate analysis was performed to identify the significantly different variable in the baseline clinical and laboratory features between the two groups. These variables were incorporated for multivariate logistic and linear regression analysis to examine the relationship between the binary and continuous variables to identify those that could significantly differentiate the 2 groups. Each variables were assigned scores based on the strength of association in the multivariate analysis (stronger the association higher the score). Different models were developed using the score and ROC-AUC was generated and compared. The best fit model was identified.

Results: 177 cases of scrub typhus and 193 cases of dengue were included in the prediction model. Dengue was taken as the outcome variable where higher scores favored dengue. From the multivariate logistic analysis, the six variables which could discriminate between ST and Dengue were oxygen saturation (>90%, <90%), age (>40 years and less than 40 years), total WBC count (<4000, 4001 – 7000 and >7000 cells/cu mm), hemoglobin (<14 and > 14 gm %), total bilirubin (<2 and >2mg%), SGOT (>200 and < 200 IU/dL) and altered sensorium (present or absent). 6 scoring models were developed, giving odds based weightage and were explored to ascertain the model with best fit.

The area under the ROC curve (95 CI) for model 1 to 6 are 0.83 (0.78 - 0.88); 0.84 (0.79-0.89); 0.79 (0.73-0.82); 0.77 (0.71 – 0.83); 0.80 (0.75 – 0.86); 0.80 (0.75 – 0.86) respectively (Figure 1 and table 1).

Thus model 2 is a simple scoring system that is found to have good diagnostic accuracy and can be used to differentiate dengue from scrub typhus. At the cut-off score of 13, the sensitivity and specificity to diagnose dengue was 85% and 77% respectively.
Conclusions

This is the first study to develop a scoring model to differentiate between scrub typhus and dengue. We developed a CSSD score which is based on simple clinical and laboratory investigations and is appropriate for resource-constrained hospitals in tropical countries to distinguish between dengue and scrub typhus. Further research is needed to validate this scoring system.

Figure 1. Receiver operating characteristic curve demonstrating the area under the curve for the 6 models to differentiate between dengue febrile illness and scrub typhus.
<table>
<thead>
<tr>
<th>Variables</th>
<th>Category</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
<th>Model 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>≤ 30 years</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>1</td>
<td>-</td>
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<tr>
<td></td>
<td>&gt; 30 years</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>SpO₂</td>
<td>&gt; 90%</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>≤ 90%</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>&gt; 14 gm%</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>≤ 14 gm%</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>-</td>
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<td>0</td>
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<tr>
<td>Total WBC count</td>
<td>≤ 4000</td>
<td>19</td>
<td>7</td>
<td>1</td>
<td>18</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4001 - 7000</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>1</td>
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<td></td>
<td>&gt; 7000</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SGOT</td>
<td>&gt; 200 IU/dL</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>≤ 200 IU/dL</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>&gt; 2mg%</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>≤ 2mg%</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Altered sensorium</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>CSSD SCORE</td>
<td>Minimum</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>43</td>
<td>25</td>
<td>10</td>
<td>22</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Area Under curve</td>
<td></td>
<td>0.83</td>
<td><strong>0.84</strong></td>
<td>0.79</td>
<td>0.77</td>
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<td>0.80</td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td>0.78 – 0.88</td>
<td><strong>0.79 – 0.89</strong></td>
<td>0.73 – 0.84</td>
<td>0.71 – 0.82</td>
<td>0.71 – 0.82</td>
<td><strong>0.75 – 0.86</strong></td>
</tr>
</tbody>
</table>
ABSTRACT


*1Olajubu, F. A.; 2Lawal, I.; 2Osinupebi, O. A. and 2Ajewole, J.O.

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Background: Suppressed immune system predisposes HIV/AIDS patients to urinary tract infections, thereby increasing the cost of treatment in this category of patients. To avoid the resultant complications of UTI, early detection of significant bacteriuria and adequate treatment is required. This study was therefore carried out to determine the prevalence of asymptomatic bacteriuria among symptom free patients attending IHVN clinic in Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria.

Methods: Manifestation of no symptom for UTI was the inclusion criterion into the study. One hundred and seventy one (171) HIV/AIDS volunteers were recruited into the study after obtaining their verbal consent. Another 75 patients with no recent history of UTI and HIV negative were used as the control group. A simple questionnaire was used to capture volunteers’ biodata. Mid stream urine samples were aseptically collected into sterile containers and cultured within 2 hrs. Colony count of monoculture of \( \geq 10^5 \) cfu/ml of urine is termed significant bacteriuria. Biochemical analyses of the urine samples were equally done. Blood samples were collected for CD4 enumeration. SPSS version 16.0 was used for data analysis.

Result: The test and control groups were made of 27, 25 males and 144, 52 females respectively. Thirty-five urine samples (20.5%) of the test group and 4.0% of the control group yielded significant bacteriuria. \textit{Staphylococcus aureus} was the most prevalent bacterium, closely followed by \textit{Escherichia coli} (23%). Among the isolates, 23(65.7) were sensitive to ofloxacin and resistant to cotrimoxazole. The mean CD4 counts were 115± 3.7 and 375± 1.8 for the test and control groups respectively.

Conclusion: The prevalence rate was 20.5%, however, 65.7% of the isolates were resistant to cotrimoxazole. The study revealed the need to urgently review the prophylactic use of cotrimoxazole among the studied population. The team therefore recommend urine culture as a routine for all HIV/AIDS patients at regular intervals.

Key words: Asymptomatic bacteriuria, Prevalence, UTI, HIV/AIDS, Sagamu.
Changing Clinical Characteristics of Pulmonary Tuberculosis in an Acute-Care General Hospital in Japan

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2)Tokyo Women’s Medical University, Tokyo, Japan

Background: Generally, pulmonary tuberculosis (TB) is suspected in patients presenting with respiratory symptoms such as cough, dyspnoea, and haemoptysis; however, most known clinical features are reported by specialised TB facilities, whereas data from non-specialised facilities are limited. The study objective was to examine the characteristics of patients positive for Mycobacterium tuberculosis at the initial visit in an acute-care general hospital without a TB clinic and to determine whether the situation has changed over time.

Methods: We retrospectively reviewed the mycobacteriology results and medical records of patients who presented at our institution between 1 October 2005 and 31 May 2014 in a single center study. Patients older than 18 years who visited the outpatient clinic and showed positivity for M. tuberculosis on culture of a respiratory tract specimen (sputum, gastric juice, or bronchoalveolar lavage fluid) were eligible. Hospitalised patients, referred patients with suspected pulmonary TB, and those with abnormal chest radiography findings in health check-ups were excluded. Data on demographic variables (age and sex) and TB-related complaints (cough, fever, night sweats, weight loss, haemoptysis, and chest pain) were collected.

We examined the changes in these variables over time by dividing patients into three groups according to the date of presentation: first group (1 August 2005 to 31 December 2007), second group (1 January 2008 to 31 October 2010) and third group (1 January 2011 to 31 May 2014). Data were analysed using Fisher’s exact test and multivariate logistic regression analysis. A two-tailed p value of <0.05 indicated statistical significance.

Results: During the 9-year study period, 77 were eligible. 16 patients were included in the first group, 24 patients in the second group, and 37 patients in the third group. The three groups did not differ significantly in terms of age (mean, 59.5, 61.0 and 58.0 years, respectively) and the male-to-female ratio (9:7, 7:1 and 28:9, respectively). The two most common chief complaints in these groups were fever (12.5%, 33.3% and 18.9%, respectively), and cough (56.3%, 20.8% and 24.3%, respectively). Among these three groups, patients presenting cough as their chief complaint decreased over time (p=0.00249, OR: 0.4547, 95% CI: 0.2283-0.9056).

Conclusion: Although patients with typical pulmonary TB predominantly present with respiratory symptoms,
our findings suggest that atypical cases lacking cough as a chief complaint at the initial visit are increasing in primary care settings. Therefore, pulmonary TB should be a differential diagnosis even in patients with fewer respiratory symptoms.
Epidemiology of Multi-Drug Resistant Enterobacteriaceae In A Community Hospital

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2Department of Medicine, National University Hospital, Singapore
3Department of Laboratory Medicine, National University Hospital, Singapore
4Department of Laboratory Medicine, Alexandra Hospital, Singapore
5Department of Medicine, Alexandra Hospital, Singapore

Background: Increasing resistance in gram-negative bacilli is a worldwide phenomenon including Singapore. With the emergence of carbapenem resistant Enterobacteriaceae, there are very few treatment options open for patients infected with these organism. Little is known about the epidemiology of multi-resistant gram-negative bacilli in community hospitals in Asia. We reviewed the epidemiology of multi-drug resistant Escherichia coli in a community hospital in Singapore.

Methods: We retrospectively reviewed all the microbiology records of Alexandra Hospital from 1Aug2010 to 1Aug2012. Non-duplicate isolates of clinical specimens obtained from routine care positive for Escherichia coli were reviewed. Susceptibility testing was performed manually using standard methodology. We defined Multi-Drug Resistant Organism (MDRO) as resistant to three of the four classes, namely Fluoroquinolones, 3rd Generation Cephalosporin, Aminoglycosides or Trimethoprim-sulphamethoxazole. We obtained the patient profile from the electronic patient medical records of Alexandra Hospital to determine the risk factors for MDR E. coli. The study was approved by the local ethics committee.

Results: 439 distinct clinical cultures were positive for E. coli during the study period. These came from 377 patients with 401 admissions. Of the admissions, 128(31.9 %) of the episodes were in male patients. The mean age was 70.8±18.3. 69(15.7%) of the 439 isolates were MDRO. Overall, 58(13.2%) of 439 isolates were bloodstream infections but only 6(10.3%) of those 58 isolates were MDRO. In contrast, 319(72.7%) of 439 isolates were from urine samples of which 54 (17.4%) of these 319 isolates from urine were MDRO. Mortality was similar in both MDRO (4.3%, 3 of 69 MDRO isolates) and non-MDRO infected patients (4.6%, 17 of 370 non-MDRO isolates) as was length of hospital stay for those with MDRO (10.2±9.9) and non-MDRO (10.3±14.5) E. coli. However, patients with MDRO cultures had more previous hospitalizations (5.6±8.5) than those with non-MDRO cultures (2.8±4.6). These were more likely to be more recent admissions (4.1±9.3 months prior to the index admission) compared to non-MDRO (13.7±19.6 months). Other risk factors identified for MDRO E. coli infection were male gender (OR=2.17 (1.29 to 3.65), p= 0.004) and abnormal albumin level (OR= 2.60 (1.27 to 5.31), p=0.0099) which is probably a marker of chronic ill health.

Conclusion: In our community hospital, the urine appears to be the major source of MDRO Enterobacteriaceae. Chronically ill patients with multiple admissions are also at risk and this should be the focus for infection control efforts.
Mean inhibitory concentration (MIC) and clinical significance of multidrug resistant *Acinetobacter* spp. isolates in a tertiary care hospital in Malaysia

*PS Wong¹, SH Hawa², KN Leong¹, TS Chow¹

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²Microbiology Unit, Department of Pathology, Penang Hospital, Malaysia.

Background: *Acinetobacter* has emerged from an organism of questionable pathogenicity to an infectious agent of importance to hospitals worldwide. Incidence of multidrug resistant *Acinetobacter* spp. has increased over the years. This study looked at the epidemiology, clinical significance, antimicrobial susceptibility pattern and the treatment regime for infections caused by *Acinetobacter* spp.

Methods: We performed a prospective observational study on patients who had multidrug resistant *Acinetobacter* spp. isolated from tracheal aspirates (from intensive care units (ICU) and high dependency units (HDU) only) and sterile sites from November 2011 to May 2012 in Penang Hospital, Malaysia. The MIC of various antimicrobials for multidrug resistant *Acinetobacter* spp. was tested using the E-test.

Results: One hundred and sixteen isolates were collected and clinical outcome were analysed for 106 isolates (72 from tracheal aspirates, 30 from blood and 4 from cerebral spinal fluid). These isolates were collected from 88 patients (58 males and 30 females, median age of 50 years). Sixty-six patients were from General ICU, 8 patients from Surgical HDU, 8 patients from Neurosurgical ICU, 4 patients from Coronary Care Unit and 1 patient each from neurosurgical HDU and orthopaedic ward respectively. Forty-seven isolates (65.3%) from tracheal secretions were pathogenic while 25 isolates (34.7%) were determined to be colonizers. Six (20%) out of 30 episodes of bacteraemia were not treated. All cerebral spinal fluid isolates were treated. Twenty-six patients (41.9%) were treated with colistin-based combination regime and 36 patients (58.1%) were treated with monotherapy. Twenty-two patients died within 2 weeks of a positive culture with nine deaths (10.2%) attributed to multidrug resistant *Acinetobacter* spp. infections. Median length of stay in hospital was 36 days. All the isolates were resistant to piperacillin-tazobactam, imipenem and meropenem. 2 isolates were sensitive to ampicillin-sulbactam and 9 isolates were sensitive to cefoperazone-sulbactam. All the isolates were sensitive to colistin.

Conclusion: *Acinetobacters* spp. isolates from our hospital were highly resistant but remained sensitive to colistin. Further studies are warranted to determine the optimal treatment regime for these infections.
The production of siderophores in *Stenotrophomonas maltophilia*

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**BACKGROUND**

Iron is an essential factor for the growth of microorganism as it is required for every metabolic process. In human blood, iron is mostly bound together with protein called transferrin, lactoferrin and ferritin thus limit the amount of free iron. The function of those proteins in blood is to trap free iron in the serum thus prevent the microbial growth. Usually, microorganism that is able to escape from the immune system in the blood will produce siderophores that will help to forage free iron from the host in order for them to survive. Siderophores are only produced when the iron is limited in the environment. These siderophores play an important role in pathogenicity for most microorganisms as it induces the secretion of other exoproteins which exhibits detrimental effect on host cells leading to life threatening infection. Galium is known to have similar psychochemical properties like Fe (III), thus Galium is mistakenly taken by bacteria to undergo redox reaction but unlike iron, Galium cannot be reduced. Theoretically, this will inhibit the growth of the bacteria as it interrupts the bacterial iron metabolism. *Stenotrophomonas maltophilia* is an environmental strain which has recently entered clinical setting and became a nosocomial pathogen. The aim of this study is to investigate and compare the production of siderophores in environmental and clinical strains.

**METHODS**

Briefly, four types of the *S. maltophilia* LMG (environment), SM13637 (ATCC), CS17 (Clinical invasive) and CS24 (clinical non-invasive) were grown in three different media: Control (Brain Heart Infusion broth), Treatment 1 (Brain Heart Infusion with the addition of 2,2 Dipyridyl as a chelater.) and Treatment 2 (Brain Heart Infusion broth with the addition of 2,2 Dipyridyl and Galium). The growth curve for each treatment was measured. Then, the siderophore production was screened qualitatively and quantitatively using modified CAS plate method and liquid CAS assay (SSC-CAS-Dip).

**RESULTS**

Based on the finding, all four strains of *S. maltophilia* produced siderophores when grown in iron limited medium. The highest production of siderophores was detected in both clinical isolates (CS17 and CS24) with 30.4% and 30.8% at 72 hours while ATCC (8%) and LMG (21%) produced little siderophores. Generally, production of siderophores started to increase after 48 hours. *S. maltophilia* that was grown in iron limited medium had longer lag phase compared to normal medium. When treated with the combination of 2, 2 dipyridyl and galium, the yellow zone of the four isolates were more intense that ranged between 10mm-14mm when compared to the treatment with 2,2 dipyridyl only which ranged from 5mm-10mm.

**CONCLUSION**

Clinical *S. maltophilia* produces high amount of siderophores compared to environmental strains. This clearly indicates that clinical strains are more virulent and siderophores production may induce the secretion of various exoprotein.
Management of community-acquired pneumonia in an Australian regional hospital

ABSTRACT:

Background: An audit was undertaken to describe the current management of hospitalized patients with community-acquired pneumonia (CAP) in an Australian regional hospital. The goals were to define the proportion of patients that are managed in compliance with the care recommended by Australian therapeutic guidelines, and to produce recommendations to improve management of CAP at a local level.

Methodology: A retrospective audit from December 2012 to November 2013 in a regional Australian hospital in North East Victoria. Adult patients above 18 years of age with a diagnosis of CAP on discharge during the study period were included. Quality indicators and outcome measures were collected. Using the pneumonia severity index (PSI), CURB-65, SMARTCOP, and CORB scores each case was assigned a severity status, and the antibiotics prescribed on hospital admission were compared with national recommendations.

Results: 107 patients with CAP were included with an average age of 71 years (range: 26-93). Average length of stay was 7 days. 34 (32%) patients were admitted to ICU with an average ICU length of stay of 5 days. Average time to first CXR was 1 hour 31 minutes, and to first antibiotic administered was 3 hours and 36 minutes. Those who had at least one investigation for CAP were 94 (88%), with 75 (70%) having blood cultures, 46 (43%) having sputum, and 45 (42%) having pneumococcal urinary antigen tested. A causative organism was found in 22 (21%) cases with Streptococcus pneumoniae being the predominant organism identified in 13 (54%). The most common antibiotic combination used was ceftriaxone and azithromycin in 60 (56%) patients. 62 (58%) cases treated were non-consistent with the guidelines. The average number of days of IV antibiotics was 4.3 days (mean=4 days), with 67 (63%) switched to oral therapy appropriately. The average number of extra IV antibiotic therapy of those not switched on time was 3.3 days (median=2 days). 15 (14%) patients suffered from acute myocardial infarction defined as a rise in troponin levels >0.05 in association with CAP, 15 (14%) had acute pulmonary oedema, and 9 (8.4%) developed a new arrhythmia (atrial fibrillation). Patients who developed ARDS were 3 (2.8%), patients who had deterioration of pre-existing co-morbidities were 6 (5.6%), who developed empyema and pulmonary embolism 2 (1.9%), and who had progression of CAP were 5 (4.6%). There were 4 (3.7%) who died during admission, 2 (2%) at 30 days and 3 (3%) at 6 months.

Conclusions: In an Australian regional hospital, ceftriaxone and azithromycin was the predominant combination used at 56%. Mild CAP was about 8 times more likely to be treated as severe CAP (OR=8.2 [95% CI: 1.7 to 40.3] p<0.009). There is a need for a simple yet effective strategy to be introduced to rationalise treatment and investigation of CAP in this setting.
Causative agents of blood stream infections in clinically suspected dengue patients at a Tertiary Care Hospital in Sri Lanka

N Senanayake*, N Chandrasiri, V Francis

Colombo South Teaching Hospital, Sri Lanka

Background:

Dengue fever is endemic in Sri Lanka and it is associated with high morbidity and mortality. The objectives of this study were to identify the causative organisms and the antibiotic susceptibility pattern of blood stream infections in clinically suspected dengue patients at the Colombo South Teaching Hospital (CSTH), Sri Lanka.

Method:

Blood samples received at the Department of Microbiology, CSTH from 1st December 2013 to 1st August 2014 were processed manually according to the standard operating procedures. Blood samples were incubated overnight at 35°C-37°C aerobically and were plated on blood, McConkey and chocolate agar plates. These plates were incubated overnight at 35°C-37°C aerobically. The isolates were initially identified by Gram staining and colony characteristics. Gram positive isolates were further identified by catalase, slide and tube coagulase tests. Lactose fermentation (LF) or non-lactose fermentation (NLF) of Gram negative isolates were recorded. The NLF isolates were further identified by oxidase, urease and KIA tests. Antibiotic susceptibility tests were carried out using Clinical Laboratory Standard Institute (CLSI) guidelines.

Results:

A total of 4277 blood samples were received during this period. Of the samples 309(7.22%) were received from clinically suspected dengue patients. Of the 309 samples 45(14.56%) blood samples yielded clinically significant pure bacterial growths. Of these isolates there were 21(46.67%) *Acinetobacter* spp., 9(20%) LF Coliforms, 5(11.11%) *S.aureus*, 4(8.89%) NLF Coliforms, 4(8.89%) *Pseudomonas* spp. and 2(4.44%) coagulase negative staphylococci (CNS) spp.

The percentages of the *Acinetobacter* and *Psuedomonas* spp. isolates that were sensitive to the tested antibiotics were as follows:

<table>
<thead>
<tr>
<th>Isolate</th>
<th>CAZ</th>
<th>CIP</th>
<th>SXT</th>
<th>CN</th>
<th>AK</th>
<th>NET</th>
<th>MEP</th>
<th>IMP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter</em> spp.</td>
<td>80.95</td>
<td>85.71</td>
<td>80.95</td>
<td>76.19</td>
<td>85.71</td>
<td>85.71</td>
<td>95.23</td>
<td>95.23</td>
</tr>
<tr>
<td><em>Psuedomonas</em> spp.</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>


The percentages of the LF and NLF Coliform isolates that were sensitive to the tested antibiotics were as follows:

<table>
<thead>
<tr>
<th>Isolate</th>
<th>AMP</th>
<th>AMC</th>
<th>SXT</th>
<th>CIP</th>
<th>CTX</th>
<th>CN</th>
<th>NET</th>
<th>AK</th>
<th>MEP</th>
<th>IMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF Coliforms</td>
<td>55.55</td>
<td>55.55</td>
<td>66.66</td>
<td>55.55</td>
<td>55.55</td>
<td>44.44</td>
<td>77.77</td>
<td>77.77</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NLF Coliforms</td>
<td>25</td>
<td>25</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
The Methicillin sensitivity of *Staphylococcus aureus* and CNS were 80% and 50% respectively.

**Conclusion:**
Most of the blood stream infections in clinically suspected dengue patients were caused by *Acinetobacter* spp. and coliforms. *Acinetobacter* spp. showed the highest susceptibility to Meropenem, Imipenem, Netilmicin, Amikacin and Ceftazidime. Coliforms showed the highest sensitivity to Meropenem, Imipenem, Netilmicin and Amikacin.
Susceptibility and Multidrug Resistance among *E. coli* from Urinary Tract Infections in Asia/Pacific – SMART 2012-2013  
M. Hackel, R. Badal, S. Lob, D. Hoban  
IHMA, Inc., Schaumburg, IL, USA

**Background:** Multidrug resistance has compromised many β-lactam agents used to treat urinary tract infections (UTI). Many oral antibiotics, including quinolones and cephalosporins, may no longer have therapeutic activity against *E. coli*, the most common UTI pathogen. These data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) summarize multidrug resistance and susceptibility (%S) among *E. coli* collected recently from UTI in Asia/Pacific.

**Methods**  
3,244 UTI *E. coli* were collected at 65 sites in 13 Asia/Pacific countries in 2012-2013. Antimicrobial susceptibility was determined using the CLSI broth microdilution method and breakpoints. Multidrug-resistant (MDR) isolates were defined as resistant to ≥3 antibiotic classes. A UTI was deemed hospital-associated (HA) or community-associated (CA) if cultured ≥48 hours or <48 hours from admission, respectively.

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

<table>
<thead>
<tr>
<th>Country</th>
<th>n</th>
<th>%MDR</th>
<th>%ESBL</th>
<th>AMK</th>
<th>SAM</th>
<th>FEP</th>
<th>CTX</th>
<th>FOX</th>
<th>CAZ</th>
<th>CRO</th>
<th>CIP</th>
<th>ETP</th>
<th>IPM</th>
<th>LVX</th>
<th>TZP</th>
</tr>
</thead>
<tbody>
<tr>
<td>India*</td>
<td>115</td>
<td>49%</td>
<td>78%</td>
<td>89.6</td>
<td>21.7</td>
<td>27.8</td>
<td>20.9</td>
<td>73.9</td>
<td>21.7</td>
<td>20.9</td>
<td>20.0</td>
<td>92.2</td>
<td>94.8</td>
<td>22.6</td>
<td>77.4</td>
</tr>
<tr>
<td>China</td>
<td>1198</td>
<td>43%</td>
<td>64%</td>
<td>93.1</td>
<td>18.4</td>
<td>43.8</td>
<td>35.0</td>
<td>75.0</td>
<td>57.7</td>
<td>34.7</td>
<td>27.1</td>
<td>95.2</td>
<td>98.3</td>
<td>29.1</td>
<td>92.6</td>
</tr>
<tr>
<td>Vietnam*</td>
<td>114</td>
<td>39%</td>
<td>62%</td>
<td>95.6</td>
<td>21.1</td>
<td>36.8</td>
<td>28.1</td>
<td>69.3</td>
<td>40.4</td>
<td>28.1</td>
<td>29.8</td>
<td>96.5</td>
<td>97.4</td>
<td>30.7</td>
<td>82.5</td>
</tr>
<tr>
<td>Thailand</td>
<td>88</td>
<td>30%</td>
<td>49%</td>
<td>95.5</td>
<td>25.0</td>
<td>51.1</td>
<td>46.6</td>
<td>81.8</td>
<td>52.3</td>
<td>45.5</td>
<td>23.9</td>
<td>98.9</td>
<td>98.9</td>
<td>25.0</td>
<td>93.2</td>
</tr>
<tr>
<td>AP</td>
<td>3244</td>
<td>27%</td>
<td>42%</td>
<td>96.3</td>
<td>29.3</td>
<td>63.2</td>
<td>54.5</td>
<td>81.6</td>
<td>67.8</td>
<td>54.8</td>
<td>47.0</td>
<td>97.7</td>
<td>99.0</td>
<td>48.8</td>
<td>92.8</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>95</td>
<td>22%</td>
<td>39%</td>
<td>100</td>
<td>27.4</td>
<td>68.4</td>
<td>66.3</td>
<td>94.7</td>
<td>83.2</td>
<td>66.3</td>
<td>43.2</td>
<td>100</td>
<td>99.0</td>
<td>47.4</td>
<td>94.7</td>
</tr>
<tr>
<td>Korea, South</td>
<td>204</td>
<td>21%</td>
<td>30%</td>
<td>99.5</td>
<td>35.8</td>
<td>73.5</td>
<td>67.7</td>
<td>90.2</td>
<td>82.4</td>
<td>68.1</td>
<td>52.5</td>
<td>100</td>
<td>100</td>
<td>53.4</td>
<td>92.2</td>
</tr>
<tr>
<td>Taiwan</td>
<td>432</td>
<td>19%</td>
<td>26%</td>
<td>98.8</td>
<td>29.4</td>
<td>77.1</td>
<td>63.2</td>
<td>79.9</td>
<td>72.7</td>
<td>62.5</td>
<td>60.4</td>
<td>99.3</td>
<td>99.5</td>
<td>62.0</td>
<td>94.9</td>
</tr>
<tr>
<td>Singapore</td>
<td>115</td>
<td>18%</td>
<td>30%</td>
<td>94.8</td>
<td>34.8</td>
<td>72.2</td>
<td>63.5</td>
<td>83.5</td>
<td>67.8</td>
<td>63.5</td>
<td>44.4</td>
<td>100</td>
<td>100</td>
<td>47.0</td>
<td>88.7</td>
</tr>
<tr>
<td>Japan</td>
<td>57</td>
<td>18%</td>
<td>28%</td>
<td>100</td>
<td>45.6</td>
<td>82.5</td>
<td>68.4</td>
<td>77.2</td>
<td>79.0</td>
<td>64.9</td>
<td>24.6</td>
<td>100</td>
<td>98.3</td>
<td>29.8</td>
<td>96.5</td>
</tr>
<tr>
<td>Philippines</td>
<td>111</td>
<td>17%</td>
<td>26%</td>
<td>99.1</td>
<td>26.1</td>
<td>79.3</td>
<td>61.3</td>
<td>77.5</td>
<td>68.5</td>
<td>71.2</td>
<td>54.1</td>
<td>100</td>
<td>100</td>
<td>55.9</td>
<td>96.4</td>
</tr>
<tr>
<td>Malaysia</td>
<td>138</td>
<td>10%</td>
<td>28%</td>
<td>97.8</td>
<td>44.2</td>
<td>84.1</td>
<td>69.6</td>
<td>88.4</td>
<td>84.8</td>
<td>73.9</td>
<td>66.7</td>
<td>100</td>
<td>100</td>
<td>68.8</td>
<td>96.4</td>
</tr>
<tr>
<td>Australia</td>
<td>239</td>
<td>7%</td>
<td>14%</td>
<td>99.6</td>
<td>50.6</td>
<td>87.5</td>
<td>83.3</td>
<td>94.6</td>
<td>87.0</td>
<td>83.7</td>
<td>83.7</td>
<td>100</td>
<td>100</td>
<td>84.1</td>
<td>95.4</td>
</tr>
<tr>
<td>Kazakhstan</td>
<td>43</td>
<td>7%</td>
<td>26%</td>
<td>100</td>
<td>65.1</td>
<td>81.4</td>
<td>81.4</td>
<td>97.7</td>
<td>86.1</td>
<td>81.4</td>
<td>81.4</td>
<td>100</td>
<td>100</td>
<td>81.4</td>
<td>95.4</td>
</tr>
<tr>
<td>New Zealand</td>
<td>295</td>
<td>3%</td>
<td>8%</td>
<td>100</td>
<td>43.4</td>
<td>94.9</td>
<td>91.2</td>
<td>93.9</td>
<td>91.5</td>
<td>91.2</td>
<td>88.8</td>
<td>100</td>
<td>100</td>
<td>89.8</td>
<td>95.9</td>
</tr>
</tbody>
</table>

*Data for India and Vietnam available for 2012 only

AMK=amikacin, SAM=ampicillin-sulbactam, FEP=cefeptime, CTX=cefotaxime, FOX=cefoxitin, CAZ=ceftazidime, CRO=ceftriaxone, CIP=ciprofloxacin, ETP=ertapenem, IPM=imipenem, LVX=levofloxacin, TZP=piperacillin-tazobactam.

While distribution between CA and HA isolates was approximately equal (1,445 and 1,783, respectively; 16 unknown), multi-drug resistance in Asia/Pacific overall was significantly higher in HA (32.1%) than CA UTI (20.7%, p<0.0001, chi-square test).

**Conclusions:**

- Multidrug resistance in *E. coli* from UTI in Asia/Pacific countries ranged from 3% in New Zealand to 49% in India, with China, Vietnam and Thailand exceeding 25%.
Susceptibility to CIP and LVX was <90% overall, with <30% susceptible in India, China, Thailand and Vietnam. The usefulness of fluoroquinolones as empiric therapy for UTI in Asia/Pacific may now be diminished.

In all countries, only AMK, ETP, and IPM inhibited >90% of *E. coli* from UTI. While this reflects the good activity of these drugs against MDR isolates, reduced %S in countries with high numbers of MDR isolates are cause for concern as carbapenemases continue to spread in Asia/Pacific in association with other resistance enzymes.
Abstract 1


Zhang H†, Yang QW†, BadalRE and Xu YC†

†This author contributed equally to this study and is joint first author.

§Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China

‖International Health Management Associates, Inc., Schaumburg, Illinois, USA

Background: The epidemiological susceptibility trends of aerobic and facultative gram-negative bacilli (GNB) causing urinary tract infections (UTI) in China were monitored and resistances to 12 common antibiotics were evaluated in 2013.

Methods: 908 strains of consecutive, non-selected GNB were isolated from the urinary tracts of patients with community-associated (CA) and hospital-associated (HA) infections attending 17 centers located in 15 Chinese cities. Minimum inhibitory concentrations (MICs) were determined using dehydrated MicroScan broth microdilution panels and phenotypic identification was carried out following Clinical and Laboratory Standards Institute guidelines at a central lab in China (Peking Union Medical College).

Results: The Enterobacteriaceae comprised 87.6% (795/908) of the total isolates, with Escherichia coli (62.0%) being the most common followed by Klebsiella pneumoniae (12.8%), Proteus mirabilis (3.9%), and 12.1% non-Enterobacteriaceae including Pseudomonas aeruginosa (7.2%) and Acinetobacter baumannii (4.0%). Among the 12 antimicrobial agents tested against Enterobacteriaceae, ertapenem (92.7%) was almost same effective as amikacin (92.5%) and imipenem (91.9%), but more effective than cefoxitin (73.0%) and ceftazidime (60.9%). In addition, extended-spectrum β-lactamases (ESBL) incidence differed between HA and CA strains of E. coli (HA=65.6%, CA=53.3%) and K. pneumoniae (HA=58.0%, CA=65.4%). CA-ESBL-positive K. pneumoniae isolates were only susceptible to ertapenem, imipenem and amikacin, but whether in CA or HA patients, ESBL-positive E. coli isolates showed 93.0% susceptibility rates to ertapenem, imipenem, amikacin and piperacillin-tazobactam. The non-fermentative gram-negative bacilli showed low susceptibilities to all antibiotics tested, except P. aeruginosa to amikacin (86.2%) and piperacillin-tazobactam (80.0%).

Conclusions: Ertapenem, imipenem, amikacin and piperacillin-tazobactam were the most effective antibiotics against ESBL-producing E. coli strains (>90%). Ertapenem, imipenem and amikacin were most active against ESBL positive K. pneumoniae (72.1%–83.8%). Thenon-fermentative gram-negative bacilli showed generally low susceptibilities. This study has summarized rates of antimicrobial resistance of UTI pathogens to several commonly used drugs in China, which will assist health care practitioners in selecting the appropriate treatment for UTI.
Figure 1. Distribution of 887 Gram-negative bacilli (GNB) strains isolated from urinary tracts of patients in 15 Chinese cities.

Table 1. *In vitro* susceptibilities of Enterobacteriaceae from UTI patients in China 2013.

<table>
<thead>
<tr>
<th>Family/Genus</th>
<th>HA/CA (%)</th>
<th>AK (%)</th>
<th>A/S (%)</th>
<th>CPE (%)</th>
<th>CFT (%)</th>
<th>CAZ (%)</th>
<th>CFX (%)</th>
<th>CP (%)</th>
<th>ETP (%)</th>
<th>IMP (%)</th>
<th>LVX (%)</th>
<th>P/T (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>12.1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>90.4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>85.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. pneumoniae, ESBL</td>
<td>92.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>85.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>85.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HA = Hospital-Associated; CA = Community-Associated; AK = amikacin; A/S = ampicillin-sulbactam; CAX = ceftriaxone; CAZ = ceftazidime; CFT = cefotaxime; CFX = cefoxitin; CP = ciprofloxacin; CPE = ceftipime; ESB = extended-spectrum beta lactamase; ETP = ertapenem; IMP = imipenem; LVX = levofloxacin; P/T = piperacillin-tazobactam. Boldfaced numbers indicate susceptibility rates over
90%.
Comparison of Sponge-wipe and Sponge-stick on the Recovery of Multi-Drug Resistant Acinetobacter Bacteria from Simulated Surface.

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

Background: Multi-drug resistant Acinetobacter (MDRA) bacteria are important pathogens. They can inhabit on patients’ skin surfaces resulting in subsequent environmental contamination. Inadequate environmental decontamination had been implicated as the cause of hospital outbreaks by MDRA. To assess the level of contamination and the effect of environmental disinfection, various methods of environmental sampling were advocated. The ease of use of these sampling methods by frontline clinical staffs, as well as the microbiological investigation workflow in the laboratories, are both important aspects in the informed infection control practices. In this study, we compared the recovery of two environmental sampling methods, the sponge-wipe and the sponge-stick, by two laboratory culture methods.

Methods: Two clinical strains of MDRA were used. The bacteria were resistant to beta-lactams, aminoglycosides, fluoroquinolones and carbapenems but susceptible to colistin. Simulated hard environmental surface were made by 5% Tryptic Soy Agar (TSA) in 9cm sterile petri-dishes. The agar surfaces were lawned with 100uL of MDRA at 50 - 300 cells/100uL and allowed to dry for 10min. The surfaces were then wiped with Sponge-wipe (Polywipe ™, in 5cm x 2.5cm portions to simulate actual clinical practices) or Sponge-stick (3M ™) in a zig-zag manner to include all agar surface area. Sponge-wipes and Sponge-sticks were cultured and enumerated by two methods: (1) direct impression culture onto a 1.5% TSA, and (2) vortexing in peptone water, followed by centrifugation and culture of the pellet on 1.5% TSA (concentration method). All experiments were done in duplicates. Continuous variables were analysed by student’s t test, p value of < 0.05 is considered significant.

Results: Direct impression culture recovered significantly less MDRA than concentration method for both sponge-wipe and sponge-stick (p < 0.05). In the concentration method, the sponge-wipe (mean recovery percentage 55.6%) recovered significantly more MDRA than the sponge-stick (mean recovery percentage 21.8%, p < 0.05). MDRA colonies remaining on simulated surface (5%TSA) were similar after wiping by sponge-wipe and sponge-stick (14.5% vs 11.0% respectively, p > 0.05).

Conclusion: Recovery of MDRA from simulated surfaces is significantly better when sampled by sponge-wipe followed by concentration culture method. However, it can only recover half of the original inoculum. In the interest of economics and ease of use, a quarter-portion of the sponge-wipe can be used. The infection control practitioners and laboratory personnel should considered these factors when implementing environmental surveillance for MDRA.

(425 words)
Skin Squamous Cell Carcinoma Risk among Lung Transplant Patients Receiving Voriconazole Therapy

C. F. Neoh¹*, G. I. Snell², B. Levvey², T. Kotsimbos², C. O. Morrissey³, M. A. Slavin⁴, K. Stewart⁵ and D.C.M. Kong⁵

¹Collaborative Drug Discovery Research (CDDR) Group, Faculty of Pharmacy, Universiti Teknologi Mara, Puncak Alam, Malaysia
²Lung Transplant Service, The Alfred Hospital, Monash University, Melbourne, Australia
³Infectious Diseases Unit, The Alfred Hospital, Monash University, Melbourne, Australia
⁴Department of Infectious Diseases, Peter MacCallum Cancer Centre, Melbourne, Australia
⁵Centre for Medicine Use and Safety, Monash University, Melbourne, Australia

Background: Prolonged voriconazole therapy may be associated with skin squamous cell carcinoma (SCC) in lung transplant (LTx) setting. Our aim was to describe the extent and outcomes of new or recurrent skin SCC among LTx patients receiving voriconazole.

Methods: This retrospective cohort study was undertaken at the The Alfred, Australia. Adult LTx recipients with skin SCC who had received voriconazole between July 2003 and June 2010 were included. Medical records and histopathology reports were reviewed. Demographics, clinical characteristics, voriconazole exposure, potential risk factors for skin SCC and manifestations of the skin SCC were recorded. The Naranjo algorithm was used to determine the likelihood of skin SCC being due to voriconazole exposure.

Results: Of the 102 LTx recipients receiving voriconazole, 14 (13.7%) had at least one episode of skin SCC: seven had skin SCC during or after voriconazole exposure; three had skin SCC before commencing voriconazole therapy, which recurred or worsened subsequently; three had a history of skin SCC prior to voriconazole being prescribed but no skin SCC was noted during or after voriconazole exposure; and one had multiple skin SCCs before LTx and voriconazole use, and developed further skin SCC post-voriconazole. The Naranjo score indicated a probable association between voriconazole use and skin SCC in three patients and a possible relationship in eight.

Conclusions: Prolonged voriconazole exposure may contribute to the development, recurrence or progression of skin SCC in LTx patients. The long-term use of voriconazole should be administered with care and routine monitoring for SCCs should be performed, particularly in those at high risk of skin carcinoma.
Early diagnosis of strongyloidiasis hyperinfection syndrome using infective larval biomarkers

Khalid Jameel Kadhim Al-Zihiry *, Mahmuda Aliyu1 Zasmy Unyah1, Roslaini Abdul Majid1, Rukman Awang Hamat1, Wan Omar Abdullah1

1 Medical Parasitology and Entomology Unit, Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia
2 Department of Microbiology, College of Medicine, Thi-Qar University, Iraq

Abstract:

Strongyloides stercoralis is the most common intestinal nematode of human that infects millions of people worldwide. Infection result in asymptomatic chronic disease which may remain undetectable. However, its unique ability to proliferate within the host can cause hyperinfection syndrome and dissemination in individuals with impaired cell mediated immunity which increases the mortality rate up to 87%. Diagnosis of hyperinfective cases can be difficult to establish and entails of high level of suspicion. The aim of the current study was to identify early specific diagnostic proteins from the components of S. ratti larval Excretory/Secretory products. Products from the non-treated (control) and treated (4.4mg/kg Prednisolone) groups were analyzed using two dimensional (2D) gel electrophoresis and MALDI-TOF/TOF MS and 10 different proteins were detected with molecular weight ranging from 30-90 KDa and 4-9 Isoelectric Point (PI). The identified proteins in this study might be the specific diagnostic biomarkers for early hyperinfection of strongyloidiasis.
Differential effects of three TCM herbs on Acanthamoeba species

M Boost1, P Cho1,2, D Sze3, G Shi2, M Yap2

1 Squina Centre for Infection Control, School of Nursing; 2 School of Optometry; 3 Department of Health Technology and Informatics; The Hong Kong Polytechnic University, Hong Kong

Background

Acanthamoeba are an important cause of serious eye infections associated with non-compliant contact lens use. Use of tap water for cleaning or rinsing contact lenses or accessories increases the risk of such infections. Treatment of Acanthamoeba infections is difficult as there are few effective agents available and the outcome is frequently poor. Traditional Chinese Medicines (TCM) have been shown to have antimicrobial properties, but have not been investigated for their effects on Acanthamoeba. This study investigated effects of three herbs against three species of Acanthamoeba.

Methods

Three strains of Acanthamoeba (A. roybera, A. polyphaga, A. castellani) were cultured in peptone-yeast extract-glucose (PYG) medium. After 10-15 days, viability of trophozoites was determined by an automated Beckman Coulter (Vi-CELL Cell Viability Analyzer, Miami, FL). The cultures were centrifuged for 10 minutes at 3000 rpm at 25°C. Trophozoites were resuspended in PBS buffer and the concentration adjusted to 10^7 per ml by diluting with PBS.

Extracts of three TCM herbs (Rhizoma coptidis, Radix scutellariae, Cortex phellodendri) (Guangzhou Herb Company, China) were prepared by dissolving 500mg in 10mL 0.9% sodium chloride and heating in a water bath at 80°C for one hour. After centrifugation the supernatants were tested for amoebicidal activity, using PBS as a control. A 0.1 ml aliquot of a PBS suspension of Acanthamoeba (10^7/ml) was added to tubes containing 9.9ml of TCM extracts and a PBS control. The solutions were mixed gently and two 0.5 ml aliquots of each solution were withdrawn for enumeration and determination of viability using the Vi-cell. The remaining Acanthamoeba preparation were incubated at room temperature and assessed again after two, four and six hours. These procedures were repeated for cysts, but exposure to solutions was followed by axenic culture to determine if cysts remained viable.

Results

R. scutellariae had a strong cidal effect against trophozoites of A. roybera but negligible effects against the other Acanthamoeba species. This herb showed a mild effect against A. roybera cysts. The remaining herbs showed some activity against A. roybera trophozoites only but were ineffective against the two pathogenic Acanthamoeba species.

Conclusion

Initial results against A. roybera are promising, suggesting that R. scutellariae may have had potential for development of a treatment for Acanthamoeba infection. However, its lack of activity against pathogenic species limits its application at this stage. Further studies are necessary to understand the nature of the resistance of these pathogenic species to R. scutellariae.
Antimicrobial Susceptibilities and Serotype of *Streptococcus pneumoniae* Isolates from Elderly Patients in Malaysia

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**Background** Infectious diseases are a major cause of morbidity and mortality in the geriatric population. Pneumococcal pneumonia is the leading cause of death attributed to infectious diseases in developed countries. In Malaysia, the pneumococcal vaccination program is not compulsory. The public is less aware of the importance of pneumococcal vaccination for elderly people (above 55 years) especially for those who are immunocompromised. The main aim of this study was to determine the serotypes and antimicrobial susceptibility of the *S. pneumoniae* isolates causing infections in patients over 55 years old. The secondary aim was to identify the proper vaccination for elderly people in Malaysia.

**Materials and Methods** In this study, 250 pneumococci isolates were obtained from hospitals all over Malaysia. Seventy three (29.2%) pneumococcal isolates were from elderly patients diagnosed with respiratory illnesses. The isolates were from blood (42.5%), other sterile sites specimens (17.8%) and sputum (39.7%). The pneumococci isolates were identified and subjected to serotyping, antibiotic susceptibility and minimum inhibitory concentration (MIC) test.

**Result** The most common serotypes were 19F (34.3%), followed by 19A (16.4%), 14 (12.3%), 23F (11.0%) 18F (5.5%), 17F (4.1%), 6B (4.1%) and 8 (2.7%). The penicillin resistance was 34.2% (MIC range 128-192µg/ml) and the erythromycin resistance was 53.4% (MIC ≥256µg/ml). Penicillin resistance was noted highly in serotype 19A (8.2%) and 19F(6.8%) followed by 23F and 14 at 5.5%. The resistance to clarithromycin and azithromycin were 42.5% and 61.6%, respectively. Clindamycin and trimethoprim-sulfamethoxazole resistance is reported at 30.1% and 49.3% respectively. Multidrug resistance (MDR) was (31.5%), commonly noted among serotypes 19F (4.1%), 19A(8.2%), and 23F (4.1%). The age groups largely affected by pneumococci infection were between 65 to 74 years old, representing about 46.6% of the isolates.

**Conclusion** It can be noted that the pneumococcal isolates from elderly patients are significantly resistant to penicillin and other antimicrobials. Although serotype 19F was commonly isolated, the rise in serotype 19A, 14 and 23F is also a serious concern. Therefore, it is important to take note of the efficacy vaccine given to elderly patients. Currently PCV7 and PCV10 are the common vaccine but it does not contain serotype 19A. Thus, it is advised that PCV13 vaccine is a better option for elderly population in Malaysia. Likewise, medical practitioners should also advise elderly patients to obtain pneumococcal vaccine.
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Background: We assessed the impact of the antibiotic restriction polices on the antibiotic use and resistance patterns of the leading nosocomial pathogens in tertiary hospital care in China and Sweden.

Methods: 14 tertiary hospitals located in 14 cities around China and 7 Swedish tertiary hospitals were selected as monitoring sites of this study. Antibiotic consumption data and bacterial resistance data were extracted from the pilot hospitals. We collected data on total isolation rate (first isolation) of clinically relevant pathogenic bacteria and the isolation rate of different species of bacteria.

Statistical description were made of the usage of antibiotics, the distribution of pathogenic bacteria, the level of resistance to individual antibiotics in each hospital over time and as congregated data for all hospitals.

Correlation and regression analysis was used to investigate the factors related to the increased or the decreased levels of bacterial resistance.

Results: A radical reduction of the overall antibiotic use at Chinese tertiary hospitals after the implementation of the national special intervention activities on clinical antibiotics application could be seen. In Sweden beta-lactamase resistant penicillin and penicillins with extended spectrum were the most used groups of antibiotics, whereas in China fluoroquinolons and cephalosporins were the most frequently prescribed groups. In Swedish hospitals S. aureus, and E. coli were significantly more often isolated compared with Chinese hospitals, where significantly more infections caused by pathogens such as P. Aeruginosa and E. faecium were treated. Changes in the consumption of all given antibiotics in Chinese hospitals resulted in a significantly decreased resistance rate in several clinical pathogens during the observation period of two years. No such change was observed for the Swedish hospitals.

Conclusions: The present study was carried out at a time when Chinese health authorities launched a campaign to discourage over prescription of antibiotics, and their efforts appear to be having an effect. Following the implementation of the intensive control program in Chinese tertiary hospitals, we found that the consumption levels of parenteral antimicrobials were reduced and accompanied by a significant increase in susceptibility patterns of both Gram-positive and Gram-negative bacteria, including ESBL-producing organisms.
Abstract Title: Typhoid Fever: A Retrospective Analysis on Epidemiology and Antimicrobial Sensitivity Characteristics

Abstract ID: 353 (Antimicrobial treatment guidelines and consensus)

Authors: KUK Nathan, YOUNG Francis, SUN Ying, LING Rebecca, CHEUNG King Tung, XIAO Cecilia, LO Giselle, VO Samuel, KEEM Michael, TEDJASEPUTRA Aditya

Introduction: Typhoid fever is a systemic illness caused by the Salmonella enterica serotype Typhi. It is predominantly prevalent in resource-limited areas where poor sanitation facilitates faecal-oral transmission. With an estimated 16 to 33 million cases worldwide each year, research efforts have focused on the prevention and management of this disease. Increasing antibiotic resistance has led to an evolution of empirical therapy; however, there is minimal Australian data analysing these patterns of resistance. Therefore, this clinical audit aims to provide an insight into the epidemiology and drug resistance trends in Australia over the past 10 years, as well as assessing possible preventative measures to combat the rising disease burden.

Methods: We undertook a retrospective analysis using infectious disease and pathology records from the Austin Hospital in Melbourne. Patients with laboratory-confirmed Salmonella Typhi infection from January 2004 to December 2013 were assessed regarding demographics, travel history and clinical management. Outcomes included drug resistance, travel destination and experience with pre-travel counselling.

Results: The majority of patients travelled to visit friends and relatives with the most popular destination being the Indian subcontinent. All isolates were sensitive to ciprofloxacin prior to 2006. From 2007 onwards, 60% of isolates were resistant. Ceftriaxone remained effective throughout this study period. No patients engaged in pre-travel medical counselling and only 1 was vaccinated against typhoid.

Conclusion: Antibiotic resistance remains a critical challenge in the eradication of Salmonella Typhi. Despite the immense financial cost of treatment, few effective
measures have been implemented to combat this situation. Travel counselling provides an efficient and accessible means for promoting preventative measures such as sanitation awareness and vaccinations; with an emphasis on those visiting friends and relatives. Further research on antibiotic resistance patterns and identification of populations at risk will also increase the efficacy of current treatment regimens.
The effects of ethanol extract and essential oil of red betel vine (Piper crocatum) leaves 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 an laboratory 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
**Klebsiella pneumoniae liver abscess in Singapore**

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**Background**

*Klebsiella pneumoniae* is an emerging cause of liver abscess, well reported in Taiwan and elsewhere in Asia. Its epidemiology in Singapore is less well defined. We report here preliminary findings from a prospective multicentre study on *K pneumoniae* liver abscess in Singapore.

**Methods**

Cases were identified either by positive blood or liver abscess fluid culture at the National University, Singapore General and Tan Tock Seng Hospitals, Singapore. *K pneumoniae* identification and antibiotic susceptibility were performed according to Clinical Laboratory Standards Institute criteria. Detailed data on demographic features, signs and symptoms, laboratory, radiographic and antibiotic susceptibility results were extracted.

**Results**

From October 9th 2013 to July 16th 2014, 557 cases of *K pneumoniae* bacteraemia were identified from the three hospitals. Among these, 85 cases of liver abscess were identified on imaging. Additionally, 44 cases of liver abscess were identified by positive fluid culture alone as blood culture was negative. After informed consent, 39 patients were recruited. Of the recruited cases, 28 (71.8%) were male and median age was 59 (range, 30-81) years. No patients had eye involvement. 18 (46.2%) patients had diabetes mellitus, 5 (12.8%) had gallstones and none had prior biliary procedures. Median Charlson’s score was 1 (range, 0-3). 5 (12.8%) required intensive care admission. Pneumonia was noted on chest X-ray in 2 (5.1%). Abscesses involved the right hepatic lobe in 23 (59.0%), left lobe in 9 (23.1%) and both in 7 (17.9%) cases. Median abscess size was 5.8 (range, 1-14) cm. Drainage was performed in 21 (53.8%). All *K pneumoniae* isolates were fully susceptible to ceftriaxone, ciprofloxacin, cotrimoxazole and ertapenem. All patients were treated according to antibiotic susceptibility and survived.

**Conclusions**

A significant minority of *K pneumoniae* bacteraemia in Singapore is associated with liver abscess. Diabetes mellitus is commonly but not always associated with this condition and endophthalmitis is uncommon. Clinical outcome is favourable with appropriate management.
Global Profiling of Multi-Drug Resistant (MDR) *Enterobacteriaceae* (TEST Program 2013)

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**Background:** Multidrug Resistant (MDR) *Enterobacteriaceae* have become a global problem. Multiple resistance mechanisms are often carried on plasmids and transposons which can provide isolates with resistance to several important classes. These mobile genetic elements rapidly disseminate into other species and can become regionally endemic. Global data from The Tigecycline Evaluation Surveillance Trial (TEST) program were analyzed to evaluate the susceptibility profiles of MDR *Enterobacteriaceae*.

**Methods:** During 2013, 9,892 isolates of *Enterobacteriaceae* from four regions were tested for susceptibility by broth microdilution and CLSI guidelines. Regions included Africa/Middle East (AFME), Europe (EU) and North (NA) and Latin (LA) America. MDR was defined as resistance to drugs from three or more different antimicrobial classes.

**Results:** Among this worldwide collection of *Enterobacteriaceae* isolates, 2,426 (24.5%) had a MDR phenotype. The rank order of MDR by region was LA (44.8%) >AFME (38.3%) > EU (26.3%) > NA (17.4%). By species, 34.5, 30.4, 25.6, 21.0, and 15.8% of *E. cloacae*, *E. aerogenes*, *K. pneumoniae*, *E. coli*, and *S. marcescens* were MDR. The individual antimicrobial profiles for all *Enterobacteriaceae* and the MDR population were as follow:

<table>
<thead>
<tr>
<th>Drug</th>
<th>All <em>Enterobacteriaceae</em> (9,892)</th>
<th>MDR (2,426)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%S</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Amikacin</td>
<td>98.5</td>
<td>2</td>
</tr>
<tr>
<td>Cefepime</td>
<td>88.3</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>80.4</td>
<td>≤1</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>81.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Meropenem</td>
<td>96.7</td>
<td>≤0.06</td>
</tr>
<tr>
<td>Pip-Tazo</td>
<td>87.1</td>
<td>2</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>97.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

FDA breakpoints used for tigecycline

**Conclusions:** The MDR rate among *Enterobacteriaceae* was much higher in AFME and LA compared to EU and NA. MDR rates of >15% were observed among five common *Enterobacteriaceae* species, with highest percentages among *Enterobacter* spp. Amikacin, meropenem and tigecycline were the most active drugs in vitro against the MDR population. The critical importance of MDR *Enterobacteriaceae* requires continual monitoring on a global scale.
Four years screening of Vancomycin-Resistant Enterococci in our hospital under circumferences of those regional spread in Kitakyushu area in Japan

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Background and Aim: Regional spread of Enterococcus faecium that showed low minimum inhibitory concentration (MIC) to Vancomycin (VCM) (4~8 mg/ml) but surely had van B gene was recognized as a serious nosocomial problem in Kitakyushu, Japan. Those strains easily become more resistant following inappropriate VCM exposure. We have been conducting Vancomycin-Resistant Enterococci (VRE) screening for 4 years since the first case was detected in August, 2010 and try to elucidate the prevalent tendency, prognosis and change of hospital-acquired rate through our effort of infection control.

Methods: Screening has been done in whole patients at admission by using VRE screening stool culture. The screening was repeated every one month later during hospitalization. Genotype was also examined if VRE positive. Pulse field gel electrophoresis (PFGE) was added in selected cases. All cases were classified to our hospital acquired (I), other care-facility acquired (II) or community acquired (III) and the latter two were considered the brought infection.

Results: Seventy-four cases (33 males and 41 females, mean age 81.1 years) (0.30%) of whole 24782 inpatients showed VRE positive. None of those positive cases developed any manifestations associated with VRE. Evaluable 71 strains were E. faecium carrying vanB. In thirty-five patients (47.3%) , VRE was detected as being originated from our hospital (I) whereas in 39 patients, as being from other origins. Particularly, in 11 cases, VRE was not from any medical facilities but from their own home (III). Number of VRE positive cases has been decreasing although it’s persistent despite of low frequency. Moreover, bringing cases have replaced our hospital-acquired cases but they haven’t been null yet. PFGE study was completed for 34strains. In the 17 isolates, the 2 isolates and another 2 isolates, PFGE patterns were analyzed to be identical (Group A, B and H). In the remaining 13 isolates, those were all distinct. Two brought cases (II) showed same PFGE patterns as those of our hospital acquired cases (Group A and B) but they also had prior hospitalization history in our hospital one year or more earlier that meant those brought VRE could not be considered utterly dissociated with our institute. Finally, 19 cases died of their own original diseases not related with VRE colonization.

Conclusions: The prevalence of E. faecium carrying vanB in Kitakyushu area has become not outstanding as seen in our hospital but still smoldering. There exist VRE capable to originate from any facilities and even community. Dense communication among each facility will be more indispensable.
Controls of the common cross-transmission urinary tract infections (UTIs) in ICU by nested DNA amplification fingerprinting (nDAF)

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Abstract:

Background: Urinary tract infections (UTIs) is the most common Nosocomial infections (NIs) (30-40%), and 20-50% of infections in intensive care units (ICUs) are UTIs. Most UTIs are asymptomatic bacteriuria. These infections are ignored and cause severe complications with high mortality. These asymptomatic patients are also potential risks in NIs controls. Restriction fragment length polymorphism (RFLP) is wildly used to trace NIs outbreak currently. But the labor- and time-consuming limit RFLP as large scale and routinely screen test. Genome DNA amplification fingerprinting (gDAF) uses random primer to generate amplified polymorphic fragment pattern of test genome, it were used for pathogens identification. gDAF is one tube reaction, it is rapid, easy and low cost method for an alternative method of NIs tracking. But gDAF required pure colony and complex pattern recognize system. These requirements limit the clinical application of gDAF.

Methods: Here, we propose a modified method, nested DNA amplification fingerprinting (nDAF). Instead of whole genome DNA, we applied two-step PCR on a specific orthologous region (ribosomal DNA operon). In first step, the orthologous region was amplified by specific primers, and then the amplified products were diluted and further amplified by random primer PCR. The first in vitro amplification avoids the requirement of colony growth. First primer of nDAF can be designed to amplified specific species from mixed samples. The choice of amplified region can also provide flexibility and designable pattern in nDAF.

Results: In this study, we collected total 30 clinical isolates from UTIs in ICU. nDAF patterns were generated from each isolates by using capillary electrophoresis (CE). We calculated the similarity distance of CE curve between each samples by using PCA and Dendrogram. The distance indicates the degree of patterns similarity between samples. With a minimum distance threshold, we defined highly similar patterns as pathogens from the same infection origin.

Conclusion: Based on the pattern similarity, we can estimate the relationship of traced pathogens. The information is useful to trace pathogens cross-transmission in the future.
**Malassezia furfur in neonatal intensive care units**

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**Background:** *Malassezia furfur* is the part of the skin microflora of neonates, but there are reports of catheter-associated infections of infants in neonatal intensive care units (NICU). Consider that the main risk factors *M. furfur* infection is intravenous lipid nutrition.

**Methods:** Study was carried out from January 2012 to April 2014. 1817 infants NICU and surgery NICU were analyzed. Microbiological monitoring included regular pharyngeal and rectal (fecal) swabs 1 time per week from 1st day of life. In case of infection symptoms additional tests were done (blood and others). For microorganism identifications MALDI-TOF MS (Bruker Daltonics) and for blood cultivation Versaterk (Trek Diagnostic Systems) and Bact/Alert (Bio-Merieux) were used. Cultivation of pharyngeal and rectal swabs was performed on standard media. First case of *M.furfur* growth was observed on MRS and Sabouraud's agar, then for cultivation of *M. furfur* we used Dixon's agar.

**Results:** *M.furfur* was identified in the GI tract from 72 (3.9%) infants (30 – surgery NICU; 42 – NICU). In 55 (76%) of them colonization of GI tract was on fluconazole prophylaxis background, in 69 (94%) *M. furfur* GI tract colonization accompanied with eosinophilia. In 3 colonized infants (4%) *M. furfur* was detected on the medical implants: 2 – in central venous catheter (CVC), 1 – in urine sample from urostomy. One of the newborn with *M. furfur* in CVC didn’t get lipid infusions, but he had clinical sepsis and negative blood culture. Newborn with *M. furfur* in urine sample, got lipid emulsions, but there was no *M.furfur* growth in CVC and this patient had no any clinical symptoms. There are no positive blood culture with *M. furfur* using automatic analysers for blood cultivation from all patients.

To assess the grow ability of *M.furfur* in the bottles for haemocultivation we inoculate 1 mL of *M.furfur* suspension with concentration $1 \times 10^5$ CFU/mL and incubate 10 days. There was no growth in.

**Conclusions:** Medical implant colonization of *M. furfur* from newborns, apparently, may not always be associated with parenteral lipid nutrition. Used automatic systems for blood cultivation (Bact/ALERT, Versa TREK) cannot detect *M.furfur* because of absence of growth inside the bottle. Therefore it requires using of additional modified media.
Epidemiology of Severe Influenza Infections and Risk Factors for Mortality in an Asian Tertiary Hospital

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Background: We describe the epidemiology of lab-confirmed influenza cases with critical illness who were admitted to a tertiary hospital in Singapore over a 3-year period, and identify factors associated with increased mortality in this population.

Methods: A retrospective study was performed using the hospital patient database to identify all inpatients with laboratory-confirmed influenza (a positive influenza PCR test) and who were critically ill during their admission (i.e. required management in an intensive care unit (ICU) and/or passed away during their admission), from 2011 to 2013. Univariate analysis was initially carried out to examine possible factors associated with increased mortality, including: patient demographic factors (age, gender, ethnic group, Charlson co-morbidity index score (CCI)); inpatient laboratory results (influenza subtype, chest X-ray results showing consolidation); and duration of treatment (length of stay (LOS) in ICU and ventilation, if applicable). Multiple logistic regression models were then applied for further assessment of the associated risk factors.

Results: A total of 188 patients were identified over the 3-year study period. Of these, 111 patients (59%) died during their admission. The study population was predominantly male (55.3%) and elderly (67.6% aged ≥ 65 years). Majority of the patients were Chinese (75%), followed by Indian (10.1%), Malay (9.6%) and other ethnic groups (5.3%). Of the influenza subtypes identified, 103 (54.8%) had the Influenza A/H3 subtype, 48 (25.5%) had Influenza B, 30 (16.0%) patients had the Influenza A/H1N1 2009 strain, and the rest had Influenza A (subtype undetermined). On application of the multivariate logistic regression model, factors independently associated with increased all-cause mortality were age ≥ 65 years (adjusted OR (AOR) 8.28; 95% CI 3.72-18.40) and signs of consolidation on chest X-ray (AOR 2.83; 95% CI 1.23-6.50) after adjusting for gender, ethnic group, CCI score and different influenza subtypes.

Conclusion: Age 65 years and above as well as confirmatory radiological evidence of pneumonia appear to be associated with increased risk of mortality among critically ill influenza patients. The findings justify recommendations for influenza vaccination for the elderly population aged 65 years and above, as well as for prompt and effective treatment of (primary or secondary) pneumonia in critically ill patients with influenza.
To evaluate the safety of the Interventions made by the ASP team in patients with Urinary Tract Infection (UTI) of Alexandra Hospital (AH)

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Alexandra Hospital (Jurong Health), Singapore.

ABSTRACT

Background: There have been concerns adopting recommendations made by the ASP team, as accepting interventions made by the ASP team may pose harm to the patients [4], [5]. The aim of this study is thus to evaluate the safety of the interventions made by the ASP team in patients with urinary tract infection (UTI) in a 500-bed restructured hospital.

Methods: This project adopts a retrospective cohort study design, studying a group of patients with UTI post ASP intervention. A univariate, followed by a multivariate analysis against composite safety endpoint analysis (which includes all-cause mortality, all-cause readmission within 3 month, recurrence and new infection) will be carried out to determine if there is an association between the various study parameters including accepting ASP intervention and the safety endpoints. A survival analysis will also be conducted to determine if accepting ASP intervention is associated with adverse outcomes.

Results: Out of the 108, 35 had an adverse outcome. 4 factors were identified to have an association with the adverse outcome, namely “prior history of UTI” (p=0.004), “abnormalities of urogenital tract” (p=0.012), “APACHE II score” (p=0.025 for Scores 0-4; p=0.005 for Scores 5-9) and “prior history of antibiotics use” (p=0.002). In the univariate analysis, accepting ASP intervention is not a factor that is associated with adverse outcomes. Survival analysis on the 35 who had an adverse outcome shows no significant difference between mean time to adverse outcomes between those who accepted and rejected ASP intervention.

Conclusion: This study has shown that accepting ASP intervention was safe and was not associated with adverse outcomes. Accepting ASP intervention also did not shorten the length of time to adverse outcomes. This study has also built a model that is fairly realistic, identifying 4 out of the 10 commonly documented factors associated with adverse outcomes, therefore showing the credibility of the results.
Different Faces of Melioidosis in Kapit District Hospital, Sarawak, Malaysia: A Report of 4 Cases & Review

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Introduction:
Melioidosis caused by Burkholderia pseudomallei is a potentially life threatening infection that is seen in Thailand, Malaysia and Northern Australia. Bacteremic spread of the disease can result in manifestation of different part of the body. Kapit district of Sarawak, Borneo, Malaysia is an endemic hot spot for melioidosis.

Objective:
The aim of this case report and review is to describe the varying sites and clinical manifestation of culture positive melioidosis in patients treated in Kapit District Hospital during the first six months of 2014.

Results:
The first patient initially presented with fever of unknown source. On day three of admission, noted cellulitic changes at his right scalp which progressed into a carbuncle. The infection spread to the right periorbital region. He was subsequently treated as septicaemic shock with right scalp carbuncle and periorbital abscess secondary to melioidosis. Incision and drainage was done. All swab, pus and blood culture grew Burkholderia pseudomallei. He completed treatment and discharged well.

The second patient initially presented with right ankle pain which showed minimal joint effusion. Blood culture however grew Burkholderia pseudomallei. Antibiotics was escalated from ceftazidime to meropenem as she developed respiratory failure with severe metabolic acidosis. USG of the right lower calf subsequently found a right intramuscular abscess where incision and drainage was done. She improved, completed treatment and discharged well.

The third patient presented with two days of left knee swelling. A left knee aspiration was done. She was not septic looking, but the knee aspiration CS grew Burkholderia pseudomallei. She undergone arthrotomy wash out and frank pus was drained. Treatment was completed and she was discharged well with no organ failure.

The fourth patient presented with two weeks history of fever. CXR showed collapsed consolidation of right upper zone. He was intubated, treated empirically as septic shock secondary to pulmonary melioidosis with respiratory failure, acute renal failure and severe metabolic acidosis. Blood culture grew Burkholderia pseudomallei. Antibiotic was escalated from ceftazidime to meropenem and he underwent urgent haemodialysis. He recovered and was discharged well.

Conclusion:
Melioidosis can present at different body sites, including blood, joint, subcutaneous tissues and intramuscular region. Contrary to literature, most patient who has melioidosis in Kapit do not have medical illness. Most Burkholderia pseudomallei in Kapit district is sensitive to gentamicin, a feature unique at this area. High suspicion in endemic area such as Kapit district is crucial in ensuring early treatment and preventing mortality.
Examination of The Host Response in The Brain Tissue During Herpes Simplex Virus Encephalitis

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Herpes simplex virus encephalitis (HSVE) continues to be one of the most devastating infections of the central nervous system despite effective antiviral treatment. The pathogenesis of the disease has not been completely studied, and little is known about the mechanisms underlying cellular death and tissue damage. Better understanding of the HSVE pathogenesis is required to develop better treatment. Post-mortem brain tissue from adult HSVE and road traffic accident cases were examined to characterize the neuropathological changes, immune activation and infiltration of inflammatory cells during herpes simplex virus type-1 (HSV-1) infection. The immuno-histochemical staining determine the type of inflammatory cells recruited during the disease. Patients with HIV encephalitis provided further control tissue. There was extensive neuropathological change and widespread necrosis in the temporal and frontal regions of the brain among the HSVE cases. The diffuse inflammation predominantly involved the grey matter (polioencephalitis) with vast majority of the central nervous system cells showed characteristic signs of lytic damage. In HSVE, infection was associated with microglial activation and infiltration by macrophages and lymphocytes. Given their abundance the inflammatory cells could certainly contribute to the damage. Since the HIV is not known to infect neurons or glial cells, the findings from the HIV encephalitis patients suggest inflammatory cells drive myelin loss. The neuropathological findings confirm previous findings for HSVE. The immuno-histochemical result of the characterization of the cellular inflammatory infiltrate in brain tissue from HSVE cases supports the thought that the inflammation can lead to the damage of the host cells during the disease process. The findings suggest that the treatments that able to reduce the inflammatory process could be a better therapeutic option in the treatment of HSVE.
Epidemiology of invasive fungal diseases in hematology patients in Asia – a prospective observational study

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Background
Invasive fungal diseases (IFDs) are a major cause of morbidity and mortality in patients with hematologic diseases. The epidemiology of IFDs in many parts of Asia is unknown. Our hypothesis is that the prevalence of IFDs—especially invasive mold infections—is high in Asian hematology centers, but not different in terms of types of IFDs compared to western institutions. We aimed to determine the prevalence and distribution of IFDs among hematology patients treated in hematology centers in Asia.

Methods
We conducted a multicenter prospective observational study between June 2012 and June 2014 where participating centers submitted both individual clinical data and population demographic data via an online database. IFDs were identified according to the EORTC-MSG 2008 definitions.

Results
Eleven institutions from 8 countries/regions participated in the study. There were 421 IFDs, comprising 120 (28.5%) possible IFDs and 301 (71.5%) probable/definite IFDs. The majority of IFDs (66.5%) were caused by Aspergillus spp., diagnosed via positive serum or bronchoalveolar lavage galactomannan. This was followed by candidemia (26.0%). The lung was the most common site of infection (74.1%). Other than Vietnam which had higher candidemia rates, institutions in other countries predominantly reported invasive aspergillosis as the most common IFD. Common associated hematological conditions included acute myeloid leukemia (AML) (49.1%), acute lymphoblastic leukemia (ALL) (20.5%), lymphoma (12.9%), and myelodysplastic syndrome (6.4%). 97 (23.1%) of patients had undergone hematopoietic stem cell transplantation (HSCT), of which the majority (79.4%) were allogeneic HSCT. A significant minority (41.0%) of patients had not received antifungal prophylaxis, whereas the most common antifungal prophylaxis regimens were fluconazole (19.3%), itraconazole (11.4%) and posaconazole (9.1%). Mortality at 30 days was 22.1%, and was distinctly higher in the subgroup with candidemia (46.2%). Six institutions had submitted demographic data, and the range of IFD breakthrough rates for various hematological conditions were: AML (7.5%-16.2%), ALL (0%-31.5%), allogeneic HSCT (5.7%-11.0%).

Conclusion
In conclusion, IFDs and IFD-associated mortality are common and high in hematology centers in Asia, with a distribution that is influenced by antifungal prophylactic strategy, similar to that seen in developed Western countries. A significant proportion of IFDs might have been preventable with the appropriate antifungal prophylaxis.
Use of a Survey to Measure Interest in Tropical Disease Education Among Health Care Practitioners in a Community-Teaching Hospital on the Mid-East Coast United States

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Introduction: Despite strong interest in tropical diseases in the USA, education in this area competes with many others for time/resources. Also, “tropical diseases” is not well-defined to some health care providers. Selecting diseases to include in teaching programs has traditionally depended on consensus of experts, practitioner surveys, or curricula suggestions from professional societies.

Background: Bryn Mawr Hospital is a 300-bed community teaching hospital with a family practice residency. It is between New York and Washington, DC, on the USA eastern coast. Endemic infections include Lyme Disease, West Nile, Babesia, and Ehrlichia. Malaria and dengue are seen intermittently. There is current concern about Ebola or Chikungunya virus infections coming to the area.

Methods: A questionnaire was prepared asking reasons for interest in tropical diseases, diseases of interest, and educational format preferred. Generalist health practitioners and nurses were targeted with a goal of 10+ surveys from each group. Answers were scored 1 (“highest interest”) to 6 (“least interest”). The survey was circulated May to August 2014.

Results: 63 forms were received: Family Practice (13), Emergency Room (9), Hospitalists (10), Nurses, self-described as “travelers” (12), Nurses “non-travelers” (12), and Internists (7). A minority of forms were incomplete/incorrectly filled out. The strongest reason for interest in tropical diseases was the chance of encountering them in practice. This was true of almost all groups, but particularly Emergency Room physicians. Academic interest and altruism also scored highly; global warming and personal experience/travel did not.

Some of the 175 topics requested appear above. Malaria was most commonly mentioned on surveys (40% of 62 first-place choices, 27% of 175 total choices). Ebola (15% first-place), and dengue (14%) also were frequently mentioned, partly due to publicity about cases in West Africa and the Caribbean in 2014. West Nile was listed by 6%, but Chikungunya was only requested by 2% of respondents despite media publicity as well. There were infrequent mentions (3 or less of 175) of TB, Chagas, MERS, yellow fever. Preferred formats varied by group, with residents ranking didactic lectures highly but most clinicians preferring case presentations. Computer-based self-teaching and printed handouts were not favored.

Conclusions: While number of responses was too few for definitive conclusions, an educational series at our institution should include malaria, Ebola, dengue, and West Nile. A revised survey giving brief descriptions of potential topics would allow curriculum refinement. A combination of case presentation and didactic lectures would be employed.
Single copy orthologous genes for microbial classification and metagenomic analysis
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Abstract:
Orthologous gene sequences have been often used for microbial identification and classification. With the development of next generation sequencing, orthologous sequences are widely used for analyzing composition of microbiome. Read count of the specific orthologous sequence is used to represent population distribution in metagenomic analysis. 16S ribosomal RNA gene (16S rRNA) is the most common orthologous genes for microbial classification. However, several drawbacks of 16S rRNA analysis have been reported. The copy number of 16S rRNA genes in each species is various. The variation will influence population estimation based on 16S rRNA counting. Microheterogeneity in 16S rRNA gene sequences within a species is common, on the other hands, some different species have identical 16S rRNA sequences. The ambiguous association reduces the accuracy of classification by using 16S rRNA sequence only. In the study, we look for alternative single-copy orthologous genes to improve the accuracy of microbiome population estimation. It can also to provide advanced evidences for microbial classification.

We collected all bacterial genome sequences from the National Center for Biotechnology Information (NCBI) genome database. Frequency of each orthologous gene was calculated, and the top-ranked 10 genes were selected as potential candidates. Sequences of candidate genes were collected from the public database. The similarity of candidate sequences was analyzed by multiple sequence alignment. We defined conserved- and variable-regions of each candidate. We designed broad-ranged primers from conserved regions of each candidate. We have performed an in silico analyzed designed-primer coverage and amplified region specificity across species. Our result provides a useful database to choose suitable target genes for precise microbiome analysis. Alternative orthologous genes information can be used to improve conventional molecular classification.
ATP: An Essential Need for Development of Intermediate Vancomycin Resistance in *Staphylococcus aureus*

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**Background**
Frequent association of VISA infections with treatment failure garners tremendous attention in the scientific communities. Furthermore, the genetic mechanism(s) leading towards the development of intermediate vancomycin resistance in *S. aureus* remains obscure due to the lack of universally defined genetic determinants to all VISA strains. This study was done to determine differential protein expression profiles of the *S. aureus* Mu50 lineage using a proteomic approach and to derive the possible vancomycin resistance regulatory pathway(s).

**Methods**
Mu50\(\Omega^1\), a vancomycin-susceptible *S. aureus* (VSSA) (vancomycin minimum inhibitory concentration (MIC) = 1 mg/L), Mu50\(\Omega^1\)-vraSm and Mu50\(\Omega^1\)-vraSm-graRm (vancomycin-intermediate *S. aureus* (VISA) with vancomycin MICs of 4 and 6 mg/L, respectively) were used in this study. These 3 strains are isogenic. Total proteins were extracted from the tested strains and identified by LC-ESI MS/MS. The resulting protein profiles were then compared between the 3 strains using Progenesis LC/MS software. Since we noticed an up-regulation of proteins associated with energy metabolism in VISA as compared to VSSA, bacterial ATP bioluminescence assay were performed to confirm the relationship between the energy component and intermediate levels of vancomycin resistance.

**Results**
Approximately 80% of differentially expressed proteins were involved in cellular metabolism. Among which all, except AacA-AphD, were up-regulated in VISA strains Mu50\(\Omega^1\)-vraSm and Mu50\(\Omega^1\)-vraSm-graRm. The molecular functions of these proteins are intriguingly associated with ATP, including ArcC2, ArcB, Drp35 and DltC. In concordance with proteomics data, significant increase of ATP concentration was found in Mu50\(\Omega^1\)-vraSm (16.00 ± 0.56 nM) and Mu50\(\Omega^1\)-vraSm-graRm (19.55 ± 1.51 nM) compared to Mu50\(\Omega^1\) (8.15 ± 0.50 nM), \((p < 0.01 \text{ and } p < 0.001 \text{ respectively})\).

**Conclusion**
Increased amount of ATP, the energy currency of cells, might be an important requirement facilitating the evolution of VSSA to VISA. Energy metabolism appears to be crucial for the development of intermediate vancomycin resistance in *S. aureus*. 
**Background**

The occurrence of obesity and type 2 diabetes among humans have been increasing. These diseases are normally caused by environmental and genetic factors. However, a new hypothesis relating gastrointestinal microbiota to the development of metabolic diseases namely obesity and type 2 diabetes are proposed. Although in recent years, a lot of studies being conducted on influences of gut microbiota in metabolic diseases, these studies focused mainly in Western populations and Asian populations especially Japan. There are few evidence of studies conducted among Malaysians. Therefore, the effect of gut microbiota in obesity and type 2 diabetes Malaysians might show a different pattern in their composition. The purpose of our research was to determine the differences in the gastrointestinal microbiota especially *Bifidobacterium* species and *Lactobacillus* species in a small group of obese and type 2 diabetes adults compared to healthy adults.

**Methods**

A total of 101 subjects were involved in the study. The data on age, height (cm) and weight (kg) were obtained from each subject and BMI (kg/m\(^2\)) was calculated. Besides, blood sample and stool sample were obtained respectively to analyze FBG (mmol/l) and gut microbiota. The qPCR method was used to quantify the copy numbers of *Bifidobacterium* and *Lactobacillus* species in each DNA sample with the genus specific 16S rRNA primers. The results was analyzed with SPSS version 21.0

**Results**

The *Bifidobacterium* was detected the highest in healthy with lowest *Lactobacillus* (R=-0.033, P=0.842). Meanwhile in obese, the copy numbers of *Lactobacillus* was increased while *Bifidobacterium* was reduced (R=-0.379, P=0.043) (P<0.05). The *Lactobacillus* and *Bifidobacterium* correlated negatively and significantly in obese subjects. In type 2 diabetes subjects, the *Bifidobacterium* was increased with a reduction in *Lactobacillus* (R=0.165, P=0.350).

**Conclusion**

It can be concluded that the differences in the *Bifidobacterium* species and *Lactobacillus* species composition influences the development of obesity and type 2 diabetes among human. However, only a small group of subjects studied in our study limits the ability of the findings to represent the Malaysian population.
SPECTRUM OF VIRAL HEPATITIS IN PATIENTS ATTENDING SUPER SPECIALITY HOSPITAL FROM NORTH INDIA


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Background: Viral hepatitis in acute and chronic form remains a major cause of morbidity and mortality in India. Exposure to Hepatitis A Virus (HAV), Hepatitis E virus (HEV), Hepatitis B Virus (HBV) and Hepatitis C Virus infection vary in different parts of the country with different socioeconomic groups. The prevalence of HAV, HEV, HBV, and HCV infection among children up to 12 years, adolescents between 13 yrs. To 18 yrs. and adults in this region attending hospital is not known.

Methods: 2892 patients (2011 Male, 881 Female) of acute and chronic liver disease with suspected viral etiology and admitted between January 2012 to July 2014 were included in the study. Clinical features of patients, routine diagnostic investigations including abdominal ultrasound were recorded. Two ml. of blood was collected, serum separated and stored at -20°C. These serum samples were tested for anti HAV IgM, anti HEV IgM, HBsAg and anti HCV by ELISA.

Results: Overall 120 children (75 Male, 45 Female, Mean age: 6.258 yrs. ±2.98 SD) of acute hepatitis and acute on chronic hepatitis were reactive for anti HAV IgM (120/2892, 4.15%) and 05 children were reactive for anti HEV IgM (0.17%). 16 children (13 Male, 03 Female) of chronic liver disease were HBsAg (0.55%) reactive and 07 children had HCV (0.24%) infection. Among adolescents 09 patients were reactive for anti HAV IgM (0.31%) and 04 adolescents had HEV (0.14%) infection. 05 adolescents were HBsAg (0.17%) reactive and 04 were having HCV (0.14%) infection. Among adult, 70 patients (58 Male, 12 Female, Mean age: 43.029 yrs. ±13.75 SD) were reactive for anti HAV IgM (2.42%), 14 patients reactive for anti HEV IgM (0.48%). 92 adult patients (73 Male, 19 Female, Mean age: 46.354 yrs. ±15.43 SD) were HBsAg (3.18%) reactive. 102 patients (82 Male, 20 Female, Mean age: 45.067 yrs. ±11.64 SD) of chronic liver disease were reactive for anti HCV (3.52%). Besides 22 patients (0.76%) had dual viral infections.

Conclusion: 4.15% of children and 2.42% of adults were suffering from HAV infection needing hospitalization indicating high infection of HAV in adults. 0.17% children and 0.48% of adults had HEV infection. HBV infection was present in 0.55% of children and 3.18% of adult patients. 0.24% of children and 3.52% of adult patients had HCV infection. Prophylactic vaccination against HAV infection is needed among children and adults from this region besides vaccination against HBV infection.
Epidemiological investigation of highly pathogenic avian influenza 2014 in South Korea.

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On 16 January 2014, a suspected case of highly pathogenic avian influenza (HPAI) was reported in Gochang, Jeollabuk-do, located in south-western part of Korea. The country had previously experienced four major epidemics of HPAI in the year of 2003/2004, 2006/2007, 2008 and 2010/2011, and the type of all the isolated viruses was H5N1. In the 2014 epidemic, however, the virus type was identified as H5N8. Considering that South Korea has very strong surveillance system to detect avian influenza, and never detected H5N8 subtype in the past in South Korea, it is assumed that the virus was introduced into Korea from foreign countries and migratory birds which fly from high latitudes during the winter is one of major suspected sources. At the initial phase of the outbreak, most farms, which were confirmed to be infected with HPAI H5N8 virus, were located in the vicinity of migratory bird habitats along the West Coast. The virus was transmitted between farms through the movement of farmers, visitors, vehicles and materials. The intravascular pathogenicity index, IVPI was 3, and the mortality rates of all viruses were 100% in chicken. But the mean death time is 4.5 days in this year, which is somewhat longer than previous epidemics of H5N1. In cases of ducks, the mortality rate was less than 20%, and the transmissibility was just 33% which is relatively low. In case of Mallard, there were no symptoms and no mortality, but its transmissibility showed 100%. In order to understand spread pattern within farm, SLIR model was developed based on basic reproductive number, doubling time and latent period and the time to die etc. The fatality of duck is not 100% and survived ducks make antibody against influenza viruses, whereas all infected chickens are die. With these models we have simulated an outbreak situation of poultry farm with 20,000 chickens or ducks. In chicken model, both of the infectivity and virulence of H5N8 showed very weak pattern compared to that of 2008 and 2010 in this simulation. In duck model, the infectivity of H5N8 showed stronger than that of 2008 H5N1, but the virulence of H5N8 showed most weak pattern. So, clinical detection of virus infection seems to not easy and takes longer time than before. This study will present the epidemiological characteristics of the 2014 epidemic of HPAI H5N8 in Korea. And the lessons learned from widespread HPAI outbreak will also be discussed.
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 AmpCbeta-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 Japan.

**Methods:** A total of 955 non-duplicate *P. mirabilis* isolated from clinical specimen in the period from 2008 to June 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, 38.3, 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 98.9% (279/282) 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), cepodoxime (2), ceftriaxone (1), cepfuamide (4), cefpirome (2), cefmetazole (16), imipenem (1), gentamicin (4), amikacin (16), and isepyamicin (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*.
Concomitant Tuberculous and Bacterial Arthritis in Chronic Tophaceous Gout
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We describe a 59-year old male with chronic tophaceous gout and pulmonary tuberculosis who have concomitant tuberculous and bacterial arthritis who presented with a four-month history of bilateral knee swelling with the right knee being worse. Left knee improved while right knee increased in swelling and eventually started draining non-foul smelling yellowish fluid with chalky material. Blood cultures were negative. Synovial fluid grew methicillin-resistant Staphylococcus aureus and tested positive for acid fast bacilli. Endotracheal aspirates tested positive for acid fast bacilli and grew Mycobacterium tuberculosis. He was treated with clindamycin, anti-Koch’s, colchicine, low-dose prednisone, febuxostat and tramadol but failed to undergo surgery. Patient demised from acute coronary syndrome.

In a systematic review of literature, this is the first report on concomitant tuberculous and bacterial arthritis in gout. This case highlights the need to explore other causes of joint swelling in a diagnosed case of chronic tophaceous gout. Concomitant TB and/or bacterial arthritis can complicate these patients. Hence, the need for microbiologic and crystal analysis of synovial fluid cannot be overly emphasized to tailor the specific management for these patients.
Antibiotic susceptibility pattern of urinary *Escherichia coli* isolates in South India

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**Background:** Urinary tract infection (UTI) is the second most common clinical indication for empirical antibiotic treatment in primary care in India. The currently recommended empirical antibiotics for treating urinary tract infections were fluoroquinolone (FQ), trimethoprim-sulfamethoxazole (SXT), nitrofurantoin (NF), fosfomycin (FOS) and aminoglycosides (AG). In view of paucity of susceptibility reports to above five antibiotics against common urinary *Escherichia coli* (*E.coli*) in India, it is difficult for the policy makers to suggest empiric antibiotics for UTI. This study was undertaken to determine susceptibility patterns for the five commonly used antibiotics against urinary *E.coli* isolates.

**Methods:** We evaluated the in vitro susceptibility of urinary *E.coli* isolates to FQ (norfloxacin), SXT, NF, FOS & AG (amikacin) for a period of 6 months from January to June 2014 in a tertiary care referral hospital in South India. The identification & antibiotic susceptibility testing were performed by the BD Phoenix TM 100 (Becton Dickinson, MD, USA) fully automated microbiology system according to CLSI guidelines.

**Results:** During study period 284 isolates of *E.coli* were identified and the susceptibility results to the five commonly prescribed antibiotics were summarized in the table 1. FQs (13.73%) has lowest sensitivity for *E.coli* followed by SXT (37.67%). NF (64.08%) & FOS (68.66%) sensitivity were similar and AG (89.78%) recorded highest sensitivity to urinary *E.coli*.

**Conclusion:** In this study major differences in the susceptibility pattern between the five commonly prescribed antibiotics against *E.coli* isolates were observed. Aminoglycosides should be the preferred antibiotic for suspected *E.coli* urinary tract infection and FQs& SXT should be avoided in the initial empirical antibiotic choice. This finding should be confirmed in a large multi-site study to create national antibiotic susceptibility data, to help antibiotic policy makers in India.
Phenotypic Screening Methods of AmpC β-lactamase and AmpC Gene Detection in Stenotrophomonas maltophilia Clinical Isolate from a General Hospital Kuala Lumpur, Malaysian.

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Abstract
AmpC β-lactamase is a resistant enzyme that can cause the development of resistance to variety of β-lactam drugs including narrow-, expanded-, and broad-spectrum cephalosporins, β-lactam-beta-lactamase inhibitor combinations, aztreonam and α-methoxy-β-lactams like cefoxitin. ampC β-lactamase is poorly inhibited by clavulanic acid. It usually found on chromosomes and also can be plasmid-mediated. Recently, there is no establish phenotypic detection methods for ampC β-lactamase. This study is focusing on the detection of ampC β-lactamase in S. maltophilia clinical isolates. 50 isolates were collected from Hospital Kuala Lumpur and identified as S. maltophilia via biochemical test and single specific primer polymerase chain reaction. All isolates were screened by disk diffusion method using ceftazidime and imipenem. Hodge test and AmpC disk test were used to identify plasmid-mediated AmpC β-lactamase. Phenotypically, 5(10%) isolates were positive for AmpC β-lactamase via disk diffusion method but no isolate (0%) detected for plasmid-mediated ampC β-lactamase. Among 5 positive isolates were run for genotypic confirmation using PCR and only 2 isolates genetically contain AmpC gene. As a conclusion, it shows that ampC in S. maltophilia is chromosomal and the production of ampC β-lactamase is low in S. maltophilia clinical isolates.

KEYWORDS: AmpC β-lactamase, Stenotrophomonas maltophilia,
Viral aetiologies of acute encephalitis syndrome in Bangladeshi children

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Acute encephalitis is the inflammation of the brain with severe consequences, such as high mortality and sequelae. Therefore requires hospitalization for treatment and follow-up. In industrialized countries the incidence varies from 5.23–7.3 cases/100,000/year with a mortality rate of 4.6–7.4%. In developing countries the situation is more severe however unknown in most countries. In industrialized countries the prevalence of encephalitis causing viruses are known as a result it helps in treatment and prevention. On the other hand in developing countries the cause is mostly unknown due to several limitations which may be responsible for higher mortality. To have a more clear picture on the aetiology of encephalitis in children of a developing countries this prospective study was performed in a tertiary care hospital in Bangladesh.

The study was conducted from April 2010 to August 2012 at Institute of Child and Mother Health Hospital (ICMH) situated in Matuail, Dhaka, Bangladesh. Hospitalized children with encephalitis were enrolled during the study period. CSF samples were subjected to routine investigations. Encephalitis-causing pathogens were detected by PCR. Nucleotide sequences of the PCR products were determined to confirm the product, and to perform phylogenetic analysis.

All targeted data were available in 96 samples. The male female ratio was 1.7:1. The age of the children ranged from 2 to 114 months. A total of 18 (18.5%) infected causes were identified, 79 (81.4%) remained unknown. Among the positive samples, 15 (83.3%) were of single virus and 3 (16.7%) were of mixed viruses. Human boca virus (HBoV), human herpes virus 1, Epstein Barr virus, human coxsackie virus B3, human echovirus 2, human parechovirus 3, and human adenovirus were identified. Phylogenetic tree showed that HBoV1 strains from encephalitis patients made a cluster. A cluster predominantly formed by HBoV1 detected in the CSF may indicate that possibly these strains are genetically similar. Full genome sequencing of strains detected in the CSF and stool may find out the differences.

Only through ongoing prospective surveillance will be apparent whether these agents will remain significant causes of encephalitis or whether they will be replaced by other emerging pathogens.
Types of multidrug resistant organisms and risk factors for mortality at a large adult tertiary-care hospital in Singapore, 2011-2013

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Background
Multidrug resistant organisms (MDROs) such as methicillin-resistant Staphylococcus aureus (MRSA), carbapenem-resistant enterobacteriaceae (CRE) and vancomycin-resistant enterococci (VRE), each has a significant impact on mortality. However, the comparative mortality risks of MDROs is not well studied. Our study aims to understand the epidemiology of MDROs in a 1500-bed adult tertiary-care hospital in Singapore, and evaluate their comparative mortality risks.

Methods
All MRDO clinical isolates from Jan 2011 through Dec 2013 were extracted from the hospital’s infection control database. Patients with a positive MDRO clinical culture for the first time during the study period were further analyzed. Demographics, hospitalization data and co-morbidities were retrieved from administrative databases. Predictors of 30-day all-cause mortality were explored.

Results
A total of 22,460 clinical isolates were included in the study. The distribution of MDROs by types of bacteria was evaluated. Of these, 95% were MRSA, 1.7% CRE, 0.6% VRE and 2.6% were others including Acinetobacter baumannii and Pseudomonas aeruginosa (Table 1). A total of 10,992 unique patient isolates were obtained. The study population was predominantly male (56%) and elderly (67.4% aged > 65 years). The median ages, years (interquartile range, IQR) were 75 (61-84) and median length of stay (LOS), days (IQR) was 10 (5-24) respectively. Five percent had a Charlson Comorbidity Index (CCI) score >3, and 40% had a length of stay (LOS) >14 days. There were 776 patients (7.1%) who died within 30 days of the clinical infection. On univariate analysis, CRE infection (OR 2.1, 95%CI 1.21-3.74), VRE infection (OR 2.6, 95%CI 1.27-5.30) and other MDRO infections (OR 5.3, 95%CI 4.00-7.03) were more likely to die within 30-days from the clinical infection. On multivariate analysis, male gender (adjusted OR (AOR) 1.2, 95% CI 1.01-1.36), age>65 years (AOR 3.0, 95% CI 2.41-3.59), CCI>3 (AOR 2.3, 95%CI 1.79-3.03) and non-MRSA infection (AOR 4.8 95%CI 3.74-6.25) were independent risk factors for 30-day all-cause mortality, after adjusting for LOS.

Conclusion
MRSA is the most common cause of MDRO infections in our patients. However, patients with non-MRSA infections were more likely to die within 30 days than those with MRSA infections, after accounting for age, gender, comorbidities, and LOS. Further studies are required to better understand the reasons for this.
Table 1: Distribution of multidrug resistant organisms by type of bacteria at a tertiary care hospital in Singapore, 2011-2013 (N=22,460)

<table>
<thead>
<tr>
<th>Organism</th>
<th>MRSA (n=21,347)</th>
<th>CRE (n=393)</th>
<th>VRE (n=131)</th>
<th>Other MDROs (n=589)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td></td>
<td></td>
<td></td>
<td>418</td>
</tr>
<tr>
<td>Acinetobacter species</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Carbapenem-Resistant Enterobacteriaceae</td>
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ABSTRACT

We report an outbreak of brucellosis on the island of Penang on the west coast of Peninsular Malaysia between March 2011 and March 2012. A total of 83 patients were diagnosed with the disease. Data of 63 patients were analysed for this report. To the authors’ knowledge this is the largest cohort of human brucellosis to be detected in Malaysia. Due to the non specific clinical symptoms the diagnosis is not often thought about when faced with a febrile patient.
Clinical Evaluation of SD BIOLINE RSV by comparison with a multiplex RT-PCR method using Seeplex RV kit

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Background: Respiratory syncytial virus (RSV) is a major important causative virus for lower respiratory traction infection. The aim of this study was to evaluate the diagnostic utility of a rapid antigen test using SD BIOLINE RSV by comparing with a multiplex reverse transcriptase PCR method using Seeplex RV kit.

Methods: A total of 551 nasopharyngeal aspirates or swabs specimens submitted for multiplex RT-PCR between October 2012 and December 2012 were included to compare with SD BIOLINE RSV. We performed both tests as manufacturer’s recommendations and analyzed diagnostic performances of a rapid antigen tests based on the results of multiplex RT-PCR.

Results: Among 551 specimens, the RSV positive rates of a rapid antigen tests and multiplex RT-PCR were 13.2% and 22.1%, respectively. The sensitivity and specificity of a rapid antigen test were 59.8% and 100.0%, respectively. Positive and negative predictive values were 100.0% and 89.7%, respectively.

Conclusion: SD BIOLINE RSV showed good diagnostic performance with easy handling and rapidity. However, we should keep in mind the possibility of false negative of SD BIOLINE RSV and the need of additional test such as RT-PCR method when viral respiratory infection is clinically suspicious.

*Presenting Author (jhsmile@inje.ac.kr)
Title: Clinical and Molecular Characteristics of Human Metapneumovirus Infection in Vietnamese Children

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Background: Human metapneumovirus (hMPV) is a viral pathogen of Acute Respiratory Infections (ARI) in young children. The virus is classified into two subgroups A and B with four genotypes (A1, A2, B1 and B2) which are thought to have an effect on disease severity. To investigate the clinical characteristics and molecular epidemiology of hMPV infection, a population based prospective surveillance was conducted at Khanh Hoa General Hospital (KHGH), central Vietnam.

Methods: All children aged <15 years from the target population admitted to KHGH with ARI from February 2007 through March 2012 were enrolled. Clinical data and nasopharyngeal swab (NPS) samples were collected. hMPV and 12 other respiratory viruses was screened by four multiplex PCR assays and genotyped by sequencing.

Results: Among enrolled 3,251 hospitalized ARI cases, 87 (3%) were positive for hMPV. All of hMPV cases was aged <5 years with overall incidence rate of 121 (98-149) per 100,000 population. Children under 1 year of age was the highest risk group with incidence rate of 361 (268-486) per 100,000 population. Children with hMPV visited the hospital earlier (p=0.014) but were hospitalized longer (p=0.011), more frequently had severe signs such as wheeze, difficulty breaking, tachypnea, and associated with IMCI pneumonia (p=0.04) and underlying disease (p<0.001). Almost all of hMPV positive belonged to genotype A2b.

Conclusion: This study supported that hMPV is one of the viral pathogens responsible for a substantial portion of annual hospitalization and severe pneumonia among pediatric ARI cases in central Vietnam.
Enhanced Surveillance for Community-Associated *Clostridium difficile* infection in Western Australia

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Background: Once considered a nosocomial infection, recent literature concerning *Clostridium difficile* infection (CDI) indicates a changing epidemiology, with growing incidence of community-associated disease. Coupled with the emergence of ‘hypervirulent’ strains (e.g. ribotype 027) causing severe disease and outbreaks in hospitalised patients, it has become necessary to investigate the burden of CDI in the community, and investigate potential reservoirs outside of the hospital environment.

Methods: The Western Australian Department of Health currently receives all reports of hospital-identified *Clostridium difficile* infections (HI-CDI) from public and private facilities participating in the state infection control surveillance program. HI-CDI includes all cases identified at a hospital, including outpatient and emergency department patients. Cases identified through primary care providers (e.g. GPs) are not included in the surveillance program. Enhanced surveillance was conducted on HI-CDI data over three years (from January 2010 – December 2012) to determine if there were a significant proportion of community-associated cases, using nationally accepted definitions.

Results: A significant proportion (24% - 30%) of the HI-CDI cases reported to WA Health in each of the years under surveillance were determined to be community associated. Given the suspected under-reporting of community-associated cases in the hospital setting, a study was proposed to determine the incidence and risk factors for community-associated CDI in WA. This will include an observational study in General Practice across the state, and a case-control study to determine likely sources of exposure in the community, including animal, food, human and environmental sources. Ethical approval has been granted for the study, and recruitment has commenced in 2014.

Conclusions: There is a substantial proportion of community-associated CDI being detected in WA hospitals, and this is likely an underestimate of the current proportions of community-associated disease. Further research is required to quantify the true proportion of CDI in the community, and to identify potential reservoirs of infection. The proposed study will provide estimates of the burden of community-associated CDI for the first time in Australia, and identify potential reservoirs which can be further investigated in order to prevent infections both inside and outside of the healthcare system.
Diarrhea is a major cause of illness and death in Bhutanese children, the morbidity rate from diarrhea in <5 years old children is 314.6/1,000 population and 13% of the death is due to diarrhea. However, information is scarce regarding childhood diarrhea. A prospective study was performed on the molecular epidemiology of rotaviruses circulating among the children under 5 years old in Bhutan. Samples of stool were collected from February 2010 to January 2011, from children attending two tertiary care hospitals in the capital Thimphu and eastern regional headquarter Mongar.

A commercial enzyme linked immunosorbent assay was used to detect rotavirus antigen in stool samples. Genomic RNA was extracted from the rotavirus positive stool samples to determine G and P types. RNA extracted by phenol-chloroform-isoamyl alcohol was used to determine the electropherotypes using polyacrylamide gel electrophoresis. Nucleotide sequence of VP7 and VP8* portion of VP4 genes were determined. A multiple sequence alignment was done using ClustalW ver. 2 and phylogenetic analyses were done by neighbor-joining method using MEGA software ver. 5.

Rotavirus positive rate was 35.8%, and composed of predominantly genotype G1 followed by G12 and G9. Only these genotypes gave rise 10 electropherotypes, indicating circulating rotavirus strains have higher diversity in Bhutan. The VP7 and VP4 genes of all genotypes clustered mainly with the neighboring countries indicating sharing common ancestor strains. The VP7 of Bhutanese G1 strains belonged to lineage 1c that is different from the vaccine strains lineage. In addition mutations were identified in the VP7 gene of G1 strains which may cause neutralization escape strains. We identified lineage 4 of P[8] genotype that are antigenically different from vaccine strains other than that, mutations were identified in Bhutanese strains of lineage 3.

Our study indicates that rotavirus is one of the major cause of diarrhea in Bhutanese children. Since the distribution of rotavirus genotypes varies from year to year therefore further study is necessary to ascertain the genotype distribution of rotavirus strains in Bhutan. A vaccine trial may be needed for Bhutanese children before implementing nationwide rotavirus vaccination program.
The Determinants Of Antiretroviral Therapy (ART) Coverage in patients with Human immunodeficiency Virus (HIV) in the selected Asian countries

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ABSTRACT

Background: According to the Joint United Nations Programme on HIV/AIDS (UNAIDS) report on the global AIDS epidemic 2013, there were an estimated 35.3 million people living with HIV worldwide. The scaling up of the uses of antiretroviral therapy (ART) has dramatically changed the number of AIDS-related death globally. However, the expanding coverage of ART varies between different countries and regions of the world. The total HIV health expenditure includes eight different AIDS spending categories (prevention, treatment, care, research, programme support etc.). The HIV expenditure due to ART is under the category of treatment. A few studies have been undertaken on the empirical analysis of ART expenditure in HIV infected patients in Asia. We were interested to know the determinants of antiretroviral therapy (ART) coverage in patients infected with HIV. In this study we made an attempt to identify the determinants of the expenditure of ART for the treatment of HIV infected people in the selected nine countries in Asia (i.e. Cambodia, China, Indonesia, Laos, Malaysia, Mongolia, Philippines, Thailand and Vietnam). Methods: The data is obtained from UNAIDS global report 2013 published by UNAIDS. The other sources of data are from World Health Statistics 2013 published by WHO, World Bank’s World Development Indicators (WDI). We employed statistical and econometric software EViews 6.0 for the analyses of data. This paper assumes that there are four factors which would determine the antiretroviral therapy coverage (ARTC) in these countries, namely, the number of people living with HIV (HIPOP), the size of the per capita gross national income (GNI), the size of the total health expenditure (HE), the HIV health expenditure (HIVHE). Results: The empirical results indicated that only one independent variable (HIVHE) have significant relationship with the ARTC in these countries. It means that the ART coverage is positively correlated with the HIV health expenditures. Conclusion: Our analyses shows when the HIV expenditure is larger, the ART coverage would expand. The result of our study will help police-makers to understand in detail how the funds are spent and to develop strategies for the funding as well as financial sustainability of AIDS programmes.
Induction of Cross-Serotype Reactive Antibodies Specific to Capsid Proteins of Rhinovirus

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Background: Rhinoviruses (RVs) represent the most important etiological agents of the common cold and responsible for about two-thirds of acute exacerbations of wheezing and asthma in both children and adults. Presently, there is no effective antiviral therapies been approved for either the prevention or treatment of diseases caused by RVs infections. Further, there are more than 100 serotypes of RVs with high sequence variability, which hinders the progression of vaccine development. Due to this variability, vaccines designed conventionally to generate neutralizing antibodies are unlikely to provide sufficient broad protection to frequent infections which occur throughout life.

Objective: In this study, we aimed at developing a pan-serotypic vaccine based on recombinant capsid proteins, which is capable of producing cross-reactive antibodies against all or most of the RVs serotypes infections.

Methods: Prior to constructing the targeted proteins, a distance matrix of the available sequences of genes encoding the RV capsid proteins (VP1, VP2, VP3 and VP4) was generated. This led to determining the relations between strains and identifying the ideal ones that are highly identical to others. From the ideal strain, four recombinant capsid proteins were constructed and produced in vitro. Upon purification, recombinant proteins were used alone or in combination to raise antibodies in rabbits and mice. The specific immunoglobulin M (IgM) and G (IgG) response to the given proteins and whole viruses were measured by enzyme-linked immunosorbent assay (ELISA).

Result: Based on bioinformatics analysis, HRV-74 was found to be an ideal strain for vaccine development. Upon alignment, VP1 amino acid sequence of HRV-74 was identical by 80% or more to 22 serotypes with a median identity of 75.5% within RV-A group. Therefore, HRV-74 has been considered as the source of recombinant capsid proteins produced in this study. The obtained antibodies reacted successfully with relevant proteins and with the whole virus particles as shown by ELISA. Anti-VP1 IgG antibodies shows high level of reactivity compared with others, whereas sera obtained from group immunized with combination of VP1 and VP4 exhibited greater reactivity. They also show strong cross-reactivity with various variant RV strains.

Conclusion: Combination of recombinant capsid proteins from the ideal strain may be considered a candidate RV vaccine.

Keywords: Rhinoviruses, Bioinformatics, cross-reactive antibodies.
Ebola virus and Marburg virus are causative agents of severe hemorrhagic fever with high mortality rates in humans and non-human primates. In 2014, there is an ongoing huge ebola outbreak in West Africa including Guinea, Liberia, Sierra Leone, and Nigeria. Rapid identification of the virus is required to prevent spread of the infection. In this study, we developed an one-step simple real-time reverse transcription-PCR assay for the rapid detection of 7 filoviruses, including 5 Ebola viruses and 2 Marburg viruses. In order to establish a rapid and sensitive method for the detection of 7 filoviruses, the TaqMan RT-PCR assay targeting the nucleoprotein genes of the 5 Ebola viruses and 2 Marburg viruses was developed, and their sensitivities and specificities were investigated. Seven DNA clones and 13 virus isolates were subjected to the real-time RT-PCR. As a result, seven DNA clones and 13 virus isolates were identified as filovirus-positive by the real-time RT-PCR. The results showed a real-time RT-PCR method with the sensitivity of 40~4,200 copies for 7 filovirus positive plasmids and 40~2,170 copies for 7 filovirus isolates, depending on each virus. This assay was specific for individual genotype detection of 7 filoviruses. In conclusion, the TaqMan RT-PCR assay developed in this study would be useful for the rapid and sensitive detection of filoviruses diagnosis in clinical samples during outbreaks.
Determination of source of Staphylococcal hand contamination of food handlers using spa typing

J Ho, M Boost, M O’Donoghue

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

Background
Although hand contamination of food handlers is a significant factor in causation of staphylococcal food poisoning, few studies have investigated transmission of *Staphylococcus aureus* in the workplace. This study aimed to determine nasal carriage status of food handlers and to relate the *spa* types present in nasal carriers to those present on the hands of workers in those establishments.

Methods
A total of 540 food handlers from 14 establishments were recruited and sampled for nasal carriage of *S. aureus* on two occasions three months apart. On the second occasion, an imprint of their fingerprints was made onto mannitol salt agar. Nasal swabs were incubated in 5% salt brain-heart infusion broth overnight before culture on *SaSelect* agar. Colonies displaying typical *S. aureus* colonial morphology on either agar were identified by latex agglutination. All *S. aureus* isolates were characterized by *spa* typing. Persistent carriers were defined as those positive for the same strain on both sampling occasions.

Results
Nasal carriage was detected in 132 (24%) subjects of whom 88 (16%) were persistent and 44 (8%) transient carriers. *S. aureus* was present on the hands of 9.8% (53/540) of subjects. Hand contamination was absent at three establishments. Four nasally-colonized subjects contaminated their hands with their own strains, of whom three were persistent carriers. Analysis of hand contaminants of the 49 non-colonized food handlers revealed 59.2% had identical *spa* types to the nasal isolates of co-workers who were persistent carriers. Only 20.4% of isolates matched those of transient carriers. The remaining hand isolates yielded unrelated *spa* types. Non-colonized workers were at a significantly higher risk of being contaminated by a strain originating from a persistently colonized co-worker than with one from a transient carrier (OR = 5.66; 95% CI 2.12-15.45; p =0.002). Spread from a single persistent carrier to hands of co-workers was observed in three sites.

Conclusions
The majority of hand contamination appeared to be associated with persistent carriers, who are likely to contaminate the environment with their strains resulting in contamination of co-workers, suggesting improvements in environmental hygiene should be coupled with continuous monitoring of hand hygiene.
Comparison of two different DNA extraction methods from urine samples

AR Uğur, El Tuncer, D Fındik*, U Arslan

Backgrounds: Besides being one of the major parts of the male and female reproductive systems, the penis and the vagina are the most common entrances for sexually transmitted infections (STIs) at sexual intercourse due to probable exchange of contagious bacteria from penis to vagina and vice versa. Molecular diagnosis is increasingly used in infectious diseases such as STIs. The aim of this study is to compare viral RNA mini kit (Qiagen, Germany) with QIAamp DNA mini kit (Qiagen, Germany) for nucleic acid extraction from the urine for bacterial identification.

Materials and Methods: Bacterial DNAs from urines of 24 healthy men were extracted by QIAamp DNA mini kit (Qiagen) and viral RNA mini kit (Qiagen, Germany) according to the instructions of the manufacturer. Bacterial specific PCRs for *L. crispatus*, *L. iners*, *A. vaginae*, BVAB 1, BVAB 2, *G. vaginalis*, *Peptinophilus* spp and *Prevotella* spp. were performed using specific primers.

Results: PCR results of both extraction methods for *L. crispatus*, *L. iners*, BVAB 1, BVAB 2, *G. vaginalis* were the same. PCR of *Peptinophilus* spp by QIAamp DNA mini kit represented seven positive results, four of which were also positive by viral RNA mini kit. Additional two positives by viral kit were negative by QIAamp DNA mini kit. In the case of *A. vaginae*, by using QIAamp DNA mini kit, eight positive PCR results were obtained and by viral kit only one positive result was obtained and that was also positive by QIAamp DNA mini kit.

Conclusion: For most of the bacteria studied, no significant PCR detection differences were observed between both DNA extraction methods. However in the case of *A. vaginae*, QIAamp DNA mini kit identified more positive PCR results than viral RNA mini kit ($p \leq 0.5$). We can conclude that although viral RNA mini kit is recommended for bacterial DNA extraction from urine samples by the manufacturer because buffer AVL supplied with this kit is said to be optimized to inactivate the numerous PCR inhibitors found in urine, according to our results, we recommend QIAamp DNA mini kit for bacterial DNA extraction from urine samples.
IDENTIFICATION OF DIPHTHERIA RISK FACTORS IN CHILDREN

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Introduction. The management of diphtheria in children, which usually late, lead us to study the risk factors of diphtheria. The particularly helpful for early diagnosis and to perform prompt treatment which will reduce mortality.

Objective. To identification the risk factors affecting the occurrence of diphtheria in children.

Methods. A retrospective cohort study was conducted to identify the risk factors of diphtheria in children. The samples were collected from medical records of dr. Wahidin Sudirohusodo hospital, Makassar of Indonesia from January 2007 to January 2014. Children suffered from swallowing pain and pseudomembrane examination, were divided into diphtheria group and non-diphtheria group.

Results. From 334 patients with swallowing pain and pseudomembrane examination, 15.6% (52) patients were included in diphtheria group and 84.4% (282) patients included in non-diphtheria group. We analysed risk factors such as age, gender, nutritional status, DPT immunization status, contact and mobility. The result of bivariate analysis revealed that there were 3 variables which associated with diphtheria: age ≥5 years (p = 0.000, COR 5.58 with 95% CI (2.43-12.82)), under nourished/poor nourished (p = 0.001, COR 2.80 with 95% CI (1.47 – 5.34)), DPT immunization not complete/no history of DPT immunization status (p = 0.000, COR 38.46 with 95% CI (17.54-83.33)), and contact (p = 0.000, COR 6.36 with 95% CI (3.04 – 13.29)).

The result of multivariate analysis revealed that there were 3 variables which associated with diphtheria: age ≥5 years (p = 0.001, AOR=5.71), DPT immunization not complete/no history of DPT immunization status (p=0.000, AOR 32.45), and contact (p=0.027, AOR=3.74).

Conclusions. Based on this study, it is concluded that the risk factors of diphtheria in children were: age ≥5 years, DPT immunization not complete/no history of DPT immunization status and contact.

Keyword : diphtheria, risk factors
Detection of *Helicobacter pylori* in tissue samples from tumors in oropharyngolaryngeal area


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Background: *Helicobacter pylori* is confirmed patogen of GIT also associated with cancerogenesis. Protein CagA is generally considered to be main pathogenicity factor. Recently, oral cavity and saliva were reported as reservoir of this bacteria, several studies reported detection of helicobacter in lymphoid tissue in oropharynx, but its eventual pathogenic activity in this area stil remains unclear. The aim of our study was to support ideas on association of *H. pylori* and cancerogenesis by detection of bacteria in deep tissue parts of tumors.

Methods: During operations of patients with tumors, surgeons were instructed to collect tissue specimen from the inner part of tumors, after washing surface using sterile buffer, into Remel M4RT transport medium and send it to the lab, where each specimen underwent nucleic acid isolation on MagnaPure Compact system. Specific DNA of *H. pylori* was detected by commercial BIORON RealLine HP Fla Test detecting fla gene. Positive specimens underwent three assay real-time multiplex PCR investigation for cagA, vacA middle region and vacA s region gene sequences (six probe genotyping) on Roche LightCycler System.

Results: Specimens from 50 patients were collected and investigated: 15 patients with tonsilar carcinoma, 16 with carcinoma of oropharynx, 4 patients with carcinoma linguae, and 15 with malignancies in hypopharyngeal and laryngeal area. *Helicobacter pylori* was detected in 41 of 50 tissue specimen (82%). Surprisingly cagA gene was detected in 7 samples only (17% of detected genotypes). In 8 of total 12 detected bacteria in tonsilar tumors identical genotype has been observed (cagA-VacAs1bm2 – 66%)

Conclusions: Colonisation of lymphoid tissue in oropharyngeal area by *Helicobacter pylori* seems to be evident. Bacteria do not stay on superficial structures, but may invade tissues (similarly as in stomach). Long-term survival of bacteria in deep tissue structures may influence immune system. Mechanisms of immune response to such infection and interaction with bacterial defense mechanisms requires detailed investigation as well as mechanisms of bacterial eventual participation in cancerogenesis.
A framework for computational fluid dynamics study of nosocomial infections

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Background: A widely accepted source of the spread of infections in hospital wards is the environmental contamination caused by the aerial dispersion of nosocomial pathogens. Numerical simulation of ventilation in hospital wards using computational fluid dynamics (CFD) tools has been frequently applied to model particle behaviour in indoor air. The results provided by these simulations are interpreted to infer bioaerosol behaviour in air, their deposition onto surfaces and the resulting risk of infection. However, there is scarce provision of guidelines in the open literature for the robust use of CFD tools necessitating the coupling of such studies with costly experimental tests for validation. The establishment of such guidelines is of paramount importance for building confidence in CFD, especially in cases where detailed experimental verification is not possible. The purpose of this study was to setup a reliable strategy for carrying out computational experiments on indoor hospital environments.

Methodology: Based on the existing practices of the simulation of indoor environments, a generic model of a hospital room was subjected to a parametric CFD study in order to analyze the sensitivity of the computations to the experimental variables. The open source CFD tool OpenFOAM was employed over a Beowulf type high performance cluster. Essential parameters which could affect the calculation of aerial dispersion in the hospital room including grid resolution, initial and boundary conditions and turbulence models were analyzed.

Results: The simulation results revealed the velocity and temperature distribution characteristics in the modeled environment. The comparison of convergence time and grid density indicated an exponential relationship between the two. This convergence time was reduced by the addition of computational nodes to the cluster, however this effect was limited to a maximum of ten nodes for the case considered. The complex relationship between these parameters was reduced to a framework defining the optimal method for domain definition, grid resolution and cluster architecture.

Conclusion: The results were used to establish a set of simple guidelines for the proper treatment of the key parameters, in order to obtain a reliable CFD calculation in the least possible time. This will assist practitioners in the design and analysis of health care facilities for the reduction of hospital acquired infections.
MOLECULAR CHARACTERISATION OF PATHOGENIC LEPTOSPIRA IN MALAYSIA USING MULTI LOCUS SEQUENCE TYPING (MLST)
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Abstract
Background: Pathogenic Leptospira species is the causative agent for leptospirosis. Leptospirosis is an emerging zoonotic disease which is increasingly being reported worldwide as well as in Malaysia. Previous molecular characterisation of pathogenic Leptospira was generally performed using culture dependent methods. The greatest challenge in performing these tests is the difficulty in the isolation of the pathogenic Leptospira especially from human clinical samples by culture technique. Thus in this study, both culture dependent and independent methods were used to characterise pathogenic Leptospira involved in human infections and also those isolated from the environment and carrier animals.

Methods: Pathogenic Leptospira isolated from human, environmental and animal samples were collected for this study. These include recent isolates and also old Malaysian isolates that were obtained from two WHO Collaborative Centres. DNA from these isolates and from blood samples of patients with leptospirosis were extracted for MLST. The MLST was performed using the six gene (adk, lipL32, lipL41, rrs, secY and icdA) method. Sequence alignments and construction of phylogenetic trees were done using the Molecular Evolutionary Genetic Analysis (Mega) version 5 software.

Results and Discussions: A total of 58 extracted DNA were analysed. However only 30 had complete MLST profiles whereby all 6 genes were amplified and sequenced. Two recent human isolates were fully amplified and sequenced. One of the human isolate which caused death due to respiratory failure had the exact sequence with the isolate from a rodent. Most of the pathogenic Leptospira genotypes with full MLST profiles were Leptospira interrogans (27/30) and 23 of the 27 Leptospira interrogans were from the serogroup Icterohaemorrhageae. A phylogenetic tree was also constructed using the rrs2 gene sequences for all the 58 sample DNAs. It was shown that this rrs2 gene sequence analysis could differentiate Leptospira into species and serotypes and could be a reliable screening for species identification when full MLST profiles were not obtained.

Conclusion: This study indicates that MLST has potential in the future characterisation of pathogenic Leptospira since it could be performed directly from clinical samples. It would be possible to study the epidemiological distributions, clinical manifestations of these pathogenic Leptospira.
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>
<thead>
<tr>
<th>Table-1 Basic Laboratory features of common AFIs in children</th>
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<tr>
<td>****</td>
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<tr>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>Total number of cases</td>
</tr>
<tr>
<td>HB (gm/dl) mean</td>
</tr>
<tr>
<td>WBC (cells/cumm) mean</td>
</tr>
<tr>
<td>Eosinopenia (Eosinophil count 0 or 1)</td>
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<tr>
<td>Platelets (mean)</td>
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<tr>
<td>CRP (mg/L)</td>
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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.
**In Vitro Evaluation of Inhalable Verapamil-Rifapentine Particles for Rapid Tuberculosis Therapy**

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**Background**

Drug efflux is a crucial resistance mechanism of *Mycobacterium tuberculosis* (*M. tb*), that prolongs treatment duration by promoting tolerance to anti-tubercular (TB) drugs and inducing intracellular bacterial survival. Compared to existing first-line anti-TB therapy, both verapamil, a FDA-approved hypertension drug that functions as an efflux inhibitor, and the first-line anti-tubercular drug, rifapentine, have separately demonstrated treatment-shortening in mouse models of TB infection. Unfortunately, anti-TB antibiotics, particularly rifamycins, have been found to induce metabolism of oral verapamil thereby reducing its bioavailability. Delivering the combination of verapamil and rifapentine directly to the lungs would be a rational approach to overcome these barriers. Additional advantages of pulmonary delivery include (1) direct targeting of infected granulomas and (2) high drug concentration at the primary site of infection. In this study, we produced an inhalable combination antibiotics formulation consisting of verapamil and crystalline rifapentine. *In vitro* aerosol performance and biological aspects including toxicity were reported.

**Methods**

A combination dry powder of verapamil and crystalline rifapentine in a mass ratio of 1:3 (based upon orally recommend dosage) with 20% L-leucine was produced using co-solvent precipitation and spray drying techniques. *In vitro* aerosol performance of the powder was assessed using a multi-stage liquid impinger (MSLI). The minimum inhibitory concentration (MIC) of the powder on *M. tb* H37Ra was determined via resazurin assay. Intracellular killing efficacy of the compounds was tested on THP-1 cells, a human monocyte cell line infected with *M. tb* H37Ra. THP-1 and human lung epithelial A549 cell lines were used to examine the *in vitro* cytotoxicity profile of the powders.

**Results**

The spray dried verapamil-rifapentine combination dry powder was found suitable for aerosolization. The total fine particle fraction (FPF<sub>total</sub>) of verapamil and rifapentine was 77.44±1.07 and 71.49±1.97, respectively. A noteworthy synergism was observed as the combination formulation had an MIC of 0.156 ng/mL, which is significantly lower than the verapamil (250 ug/mL) and rifapentine (0.625 ng/mL) alone. Rifapentine also augmented the intracellular killing of *M. tb* in the presence of verapamil. All the powders were shown an acceptable half maximal inhibitory concentration (IC<sub>50</sub>) on both THP-1 and A549 cell lines.

**Conclusion**

We have demonstrated a novel inhalable verapamil-rifapentine dry powder with good aerosol performance that shows potent activity against *M. tuberculosis* in *in vitro* models. This preliminary data warrants further in vivo studies.
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.
Blood culture plays a major diagnostic role in medicine for decades. It is critical in managing patients with infection and to direct the appropriate selection of antimicrobial therapy. One limitation of this diagnostic role is obtaining false positive results, which increase expenses and have an adverse effect on patient safety. Initial data collection in June to October 2013 has shown that, the lowest blood culture contamination rate was 2.3% and the highest rate was 7.9% with mean blood culture contamination rate as 4.45%. Mean blood culture contamination rate in our hospital was higher than recommended rate (1-3%). Factors contributing to high blood culture contamination rate in our setting were inappropriate aseptic steps, lack of knowledge and lack of adherence towards aseptic technique for blood culture taking among phlebotomists (house officer and medical officer). Several interventions were introduced to improve on aseptic technique for taking blood cultures and to increase phlebotomist knowledge and adherence towards all the aseptic steps. These include introducing guidelines on how to take blood culture focusing on aseptic steps, standardize blood culture set and change skin antisepsis to 2% chlorhexidine in 70% alcohol. Training for all phlebotomists and sisters in charge of the ward regarding new guidelines was conducted. The training includes presentation of initial survey results, briefing of new guidelines, video demonstration and hands on session for aseptic blood culture taking. We also provide a pocket guideline for blood culture collection and checklist for blood culture taking to phlebotomists. Our aim is to reduce and maintain monthly blood culture contamination rate at less than 3%. Monthly monitoring of blood culture contamination rate after intervention from November 2013 until July 2014 (9 months) revealed that the lowest rate was 1.5% and the highest rate was 2.9% with mean contamination rate of 2.23%. We conclude that, all the interventions done were able to reduce blood culture contamination rate in our setting and achieved the aim of recommended rate.