**Methods:** MLSA was done for 48 strains of VGS isolated from blood cultures of patients with IE by using concatenated sequences of the seven house-keeping genes map, pfl, pyk, ppaC, rpoB, soda, and tuf (http://www.eMLSA.net),. Nucleotide alignments and phylogenetic trees were constructed with the neighbour-joining method using single house-keeping genes and concatenated sequences of seven house-keeping genes in MEGA version 5.0.

**Results:** Analysis of 36 strains out of 48 VGS was done, since six *Streptococcus oralis* did not reach the required sequence length for the *sodA* gene when analysed by the MLSA software, and one *Streptococcus mutans* and five *Streptococcus sanguinis* did not amplify *map* gene by PCR. All the sequence clusters could be equated with recognized species and based on MLSA, twenty one were identified as *Streptococcus oralis*, six as *Streptococcus gordonii*, five as *Streptococcus sanguinis*, three as *Streptococcus parasanguinis*, and one as *Streptococcus anginosus*.

**Conclusion:** Results of MLSA revealed that the closely related species of VGS fall into well-resolved clusters when compared to single gene-based identification.

http://dx.doi.org/10.1016/j.ijid.2012.05.836

**Type: Poster Presentation**

**Genetic diversity of Malaysian methicillin resistant *Staphylococcus aureus* strains based on virulotypes, pulsed-field gel electrophoresis and PCR-RFLP of coa gene**

K.T. Lim[1],[*], Y. Abu Hanifah[2], M.Y. Mohd Yusof[2], K.-L. Thong[3]

[1] *University of Malaya, Kuala Lumpur, WP, Malaysia*
[2] *University of Malya, Kuala Lumpur, Malaysia*
[3] *Institute of Biological Sciences, Laboratory of Biomedical Science and Molecular Microbiology, University of Malaya, Kuala Lumpur, Malaysia*

**Background:** *Staphylococcus aureus* is a persistent human pathogen responsible for a variety of infections ranging from soft-tissue infections to bacteremia. The objective of this study was to determine the prevalence of a repertoire of toxin genes among Malaysian MRSA strains isolated over a four years period and the genetic relatedness of MRSA strains.

**Methods:** One hundred eighty-eight strains (2003, 2004, 2007 and 2008) of methicillin-resistant *S. aureus* (MRSA) isolated from a tertiary hospital were screened for 20 genes encoding for extracellular virulence determinant (*sea, seb, sec, sed, see, seg, seh, sei, sej, tst, eta, etb,etd*) and adhesins (*cna, etb, fnbA, fnbB, hlg, ica, sdrE*) via PCR. The genetic relatedness of these strains was determined by PFGE, PCR-RFLP of *coa* gene and *agr* grouping.

**Results:** Majority of the strains were tested positive for *efb* and *fnbA* (96% each), *ica* (78%) and *hlg* (59%) genes. A total of 101 strains were positive for at least one type of staphylococcal enterotoxin genes with *sea* being the predominant. Genes for *seb, sed, see, seh, sej, eta* and *etb* were not detected in any of the MRSA strains. The prevalence of *sea*, *sec* and *ica* among strains isolated in 2008 was increased significantly (p < 0.05) compared to 2003. Most of the strains were of *agr* type I (97.5%) followed by *agr* type II (1.2%) and *agr* type III (0.6%). Subtyping by PFGE and PCR-RFLP of coa gene produce 88 different pulsed-field profiles (F = 0.51-1.0) and 47 different patterns (F = 0.24-1.0), respectively.

**Conclusion:** No direct correlation between virulotypes, PFGE and PCR-RFLP profiles was observed. Strains with identical PFGE and PCR-RFLP profiles frequently belonged to different virulence patterns. Increase of MRSA strains with virulence factors over the years signal the potential loss of the usage of antimicrobial agents in treating MRSA infections as MRSA strains with virulence factors are normally resistant to host immune systems and other antimicrobial agents. The MRSA clinical strains from this tertiary hospital were genetically related, suggesting that few predominant clones of the species are involved in infections.

http://dx.doi.org/10.1016/j.ijid.2012.05.837

**Type: Poster Presentation**

**Aetiological agents of meningitis in Zambia: Is there a need for a pneumococcal vaccine?**

C. Lukwesa[*], J. Mwansa, R. Nakazwe

*University Teaching Hospital, Lusaka, ZA, Zambia*

**Background:** Meningitis continues to be a major cause of morbidity and mortality in Zambia. This is a problem more especially in HIV/AIDS patients and the paediatric age group. Because of the higher risk of mortality in the under five age group, the Zambian government with the aid of the World Health Organisation has an on-going surveillance of Paediatric Bacterial Meningitis (PBM) to determine the causative agents. The data is for patient management as well as policy decisions such as introduction of vaccines for immunisation against pneumonia and meningitis. The country is in the process of introducing a pneumococcal vaccine and therefore requires information on serotypes to decide on whether to introduce 7, 10, 13 or 23 valent pneumococcal vaccines.

The University Teaching Hospital Microbiology (UTH) laboratory, Lusaka, receives over two thousand Cerebral Spinal Fluid specimens for the diagnosis of meningitis. The microbiological procedures include microscopy examination (cell count, India ink and Gram staining). Samples are also cultured for bacterial and fungal pathogens and antimicrobial susceptibility testing by the Buaer-Kirby and MIC methods.

**Methods:** The UTH laboratory data for the year 2011 was reviewed to determine the main causative agents of meningitis, and the antimicrobial susceptibility. Some strains (17) of Streptococcus pneumoniawere serotyped at the National Institute of Infectious Diseases (NICD), South Africa.

**Results:** About 90% of all specimens suggest aseptic meningitis. The positive cultures included the fungal agentCryptococcus neoformans (42%)and bacterial pathogens wereStreptococcus pneumonia (37%), Neisseria meningitides (5%),Salmonella Typhi (2%)and other organisms. The pneumococcal serotypes identified included 1, 4, 6A, 6B, 7F, 10F, 15C, 19A, and 23F. More than 23% of Streptococcuspneumoniaewas isolated from the paediatric age group though all age groups were affected. The antibiotic resistance of 17strains ofS.pneumoniaetoChloramphenicol (Buaer-Kirby), Penicillin (MIC 0.12-1.00 µg/ml), and Cefotaxime (MIC≤0.5 µg/ml) were 41%, 23%, and 0% respectively.

**Conclusion:** Despite the introduction of Antiretroviral drugs (HAART), Cryptococcus continues to be a common opportunistic infection in HIV/AIDs patients in Zambia. S. pneumoniaeis the major

cause of bacterial meningitis and therefore justifies Zambia introducing a vaccine to immunise those at high risk but more strains need to be serotyped to decide on the vaccine.

**Type: Poster Presentation**

Final Abstract Number: 45.048
*Session: Bacterial Infections*
*Date: Friday, June 15, 2012*
*Time: 12:45-14:15*
*Room: Poster & Exhibition Area*

**SCC*mec* type IX in a community-acquired methicillin-resistant *Staphylococcus aureus* isolate: first report in a patient from Thailand**

A. Lulitanond [1,*], T. Ito [2], S.S. Li [2], X. Han [2], X.X. Ma [2], C. Engchanil [3], N. Jiwakanon [4], A. Chanawong [1], C. Wilailuckana [1], K. Hiramatsu [2]

[1] *Khon Kaen University, Khon Kaen, Thailand*
[2] *Graduate School of Medicine, Juntendo University, Tokyo, Japan*
[3] *Faculty of Medicine, Khon Kaen University, Thailand;, Khon Kaen, Thailand*
[4] *Veterinary Research and Development Center, Khon Kaen, Thailand*

**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is mostly associated with hospital, whereas community-acquired MRSA (CA-MRSA) infections in Thailand have been uncommon. The present study aimed to investigate 14 MRSA strains isolated from outpatients of a university hospital in Thailand.

**Methods:** Fourteen MRSA isolates were collected between September 2005 and March 2006 from outpatients of a university hospital inThailand. Minimum inhibitory concentrations (MICs) of six antimicrobial agents: vancomycin, cefazolin, oxacillin, cefoxitin, tetracycline, erythromycin and ofloxacin were determined using an agar dilution method. Genotypic studies such as SCC*mec* type, coagulase typ, *agr* type, *spa* type, multilocus sequence type (MLST) were performed using PCR and subsequent nucleotide sequences. Pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested chromosomal DNA was also performed.

**Results:** All 14 MRSA were multidrug-resistant with high MICs of cefazolin, oxacillin, cefoxitin, tetracycline and erythromycin (32->64 μg/ml), however all isolates were susceptible to vancomycin (MIC 1-2 μg/ml). Of the 14 isolates, 13 carried type III SCC*mec* and belonged to ST239, coagulase type IV, *agr* I and *spa* type-t037 but one isolate was t233. The remaining isolate (strain JCSC6690) carried a new SCC*mec* element, class C2 *mec* gene complex with *ccrA1B1*, type XIc coagulase, *agr* II, *spa* type-t337 and ST9. PFGE of the 14 isolates showed that 12 isolates gave similar bands pattern to a hospital acquired-MRSA (HA-MRSA) from the same hospital. The other isolates with *spa* type t337 and t233 showed a unique DNA profile. The Panton-Valentine Leukocidin gene was not found in these isolates. The strain JCSC6690 was isolated in March of 2006, from a 2-year-old boy underlined with atopic dermatitis, attended the hospital because of suffering from chronic impetigo at left foot. The patient achieved good recovery after receiving cefaclor. He had never been admitted in a hospital during the former year. Nucleotide sequencing of the 57 kb region at the downstream of *orfX* revealed a novel SCC*mec* carrying type I *ccr* gene complex and classC2 *mec* gene complex.

**Conclusion:** We reported a real community acquired-MRSA (CA-MRSA) from a patient in Thailand with SCC*mec* type IX - ST9 which carried class C2 *mec* gene complex and *ccrA1B1*.

**Type: Poster Presentation**

Final Abstract Number: 45.049?Session: Bacterial Infections?Date: Friday, June 15, 2012?Time: 12:45-14:15?Room: Poster & Exhibition Area

**Differential host gene expression upon exposure to live *Burkholderia cepacia* and its secretory proteins**

V. Mariappan [1,*], K.M. Vellasamy [1], J. Thimma [2], O. Hashim [2], J. Vadivelu [3]

[1] *University of Malaya, Kuala Lumour, Malaysia*
[2] *University of Malaya, Kuala Lumpur, Malaysia*
[3] *University Malaya, Kuala Lumpur, Malaysia*

**Background:** *Burkholderia cepacia* causes serious respiratory infections in immunocompromised individuals and patients with cystic fibrosis. The bacterium is known to produce virulence factors such as secretory enzymes which damage host membrane to promote invasion as well as unique pilus which is involved in adhesion and colonisation of the respiratory tract. Understanding this complex cross-talk between the host and pathogen is essential to improve understanding of an infectious disease and to identify host-defense strategies including the underlying regulatory mechanisms. The aim of this study was to investigate the transcriptional changes in the host upon exposure to live *B. cepacia* and its secretory proteins.

**Methods:** Comparison of host response to live *B. cepacia* (mid-log phase) and its secretory proteins (mid-log and early stationary phases) was performed using the Illumina HumanRef-8 microarray platform. The raw microarray data were analyzed and the web-based softwares GOTerm Finder (http://go.princeton.edu/cgi-bin/GOTermFinder) and GeneTrail (http://genetrail.bioinf.uni-sb.de/) were used to analyse significant pathways. The microarray data were validated using quantitative real-time polymerase chain reaction.

**Results:** Interaction of the human epithelial cells, A549, with live *B. cepacia* or the secretory proteins was found to differentially regulate genes that are related to metabolism, cell cycle, apoptosis and inflammatory. The host cell cycle and metabolic pathways, particularly glycolysis/glycogenesis and fatty acid metabolism were up-regulated transcriptionally. The host immune response was also found to be manipulated through the suppression of pro-inflammatory cytokines production. Additionally, the microarray analysis indicated that the host cells inhibit the apoptotic pathway during infection. Alteration of these pathways might explain the need for the host cells to survive and proliferate to sustain cell injuries caused by the secretory proteins and/or to allow prolonged survival of *B. cepacia* in the host cells. These pathogens have also been shown to modulate the epithelial bactericidal response in favour of its intracellular survival and persistence in the human host.

**Conclusion:** The differential gene expression profile of A549 cells towards *B. cepacia* infections has provided preliminary insight into the mechanisms of pathogenesis of *B. cepacia* and its secretory proteins. The microarray results permit a rational design for