International Journal of Antimicrobial Agents 45 S2 (2015) S59–S143

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Abstracts of the 10th International Symposium on Antimicrobial Agents and Resistance (ISAAR 2015)

Songdo ConvensiA, Incheon, Korea, May 14-15, 2015

Poster Presentations

May 14, 2015

P1-CM01
The value of RT-multiplex PCR and NS1 antigen detection for confirmed diagnosis of dengue hemorrhagic fever

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Background: RT-PCR and NS1 detection are recognized as the laboratory tools for diagnosis Dengue hemorrhagic fever (DHF) in the early days of disease. In Vietnam there have been few studies on the value of these two diagnostic tools applied to confirm diagnostic of DHF.
Aims of the study: Study on the value of RT-PCR and NS1 in the confirmed diagnosis of DHF.
Material and method: The objectives of the study are the adults hospitalized Cu Chi hospital with DHF suspected as the clinical diagnosis. The RT-PCR for detection and typing of Dengue were done with multiplex PCR using the type-specific primers designed by Lanciotti. NS1 detection and IgM/IgG specific Dengue were done with the rapid test kit manufactured by SD (Korea). The relevant clinical and paraclinical manifestations were also reported.
Results and discussion: 121 adult patients with DHF suspected hospitalized in Cu Chi hospital were enrolled in the study, in which 95 (78.5%) were confirmed DHF with criteria: fever + hemorrhagie (skin or mucosa) + hematology (Platelet <100.000 or Hct >47%) or RT-PCR [+]. The RT-PCR results said that the predominant type of Dengue is D2 (64%), the other types is less common (D3: 20%, D4: 13%, D1: 3%). The sensitivity of RT-PCR is 74% and specificity 100%, the sensitivity of NS1 is 85% while the specificity is 42%. IgM reached the sensitivity 93% but the specificity is as low as 36%. RT-PCR and NS1 give the sensitivity from 73% or above on the patients with 1 to 4 days of disease, but during this period of disease the IgM give the very low sensitivity (<35%). In addition, RT-PCR and NS1 can give the high possibility for confirmed diagnosis of DHF even the platelet and Hct parameter in the patients were still within the normal range. Another special good point of RT-PCR is not only detecting Dengue virus but also report the type of Dengue, and this report is the valuable key report for following up of epidemic trend of DHF in the region.
Conclusion: RT-PCR and NS1 are the high sensitivity diagnostic tool for confirmed diagnosis of DHF but NS1 is a little bit low in the specificity. IgM specific Dengue can give the high sensitivity on the patients from 4 days of disease, therefore IgM cannot seen as the goo tool for early diagnosis of DHF within the first few day of disease. In order to follow-up the epidemic trend of DHF in the region, the RT-PCR is the most valuable tool.

P1-CM02
Develop the real-time PCR using taqman probe targeted the gag gene to detect and quantify HIV1

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Background: Detection and quantification of HIV1RNA is the necessary requirement from the clinical to detect HIV infection during the window phase or to confirm the HIV infection in the baby, as well as to follow up the efficacy of the specific treatment. These requirements could be solved by the using of the IVD approved kits using PCR or bDNA technologies. However the price of these commercialized kits are very expensive, it could not be easily applied at the clinical laboratory in the existing conditions of the low-income countries like Vietnam.
Main aims: There are two main aims: (1) Prepare the molecular biology kit named HIV1 RT-TQPCR kit, to detect and quantify of the HIV1RNA based on the RT real-time PCR technology using the primers and taqman probe specific the gag gene on the HIV1 genome; (2) Evaluate the process via the limit of detection, the range of quantification, the accuracy, the specificity, the elimination of the inhibitors, and the stability of the kits as well as the sensitivity-specificity and the similarity when testing on the real samples using HIV1 Amplicors as gold standards.
Materials and methods: The "HIV1 RT-TQPCR" was prepared using the published SK462 and SK431 specific to gag gene, and the taqman probe was designed from the modification of the published probe SK102. The positive control HIV1RNA and the standards for quantification was prepared from the transcription of the plasmid pGEM-T Easy inserted with the PCR product amplified from the gag gene using primers SK462 and SK431. The internal control RNA using the same primers of the target was also prepared from the transcription of the plasmid pGEM-T Easy inserted with the PCR product amplified from the two constructed oligo with SK462 and SK431 sequence at the 5' end and the middle sequence complementary with the internal control probe. The RNA extraction kit is the [NK]RNAPREP of Nam Khoa and the cDNA synthesis kit is the [NK]cDNA synthesis kit of Nam Khoa. The limit of detection, the range of quantification, the in testing and the trans-testing reproducibility were tested on the serial dilution of HIV1RNA. The risk of the cross reaction were tested on the sera taken from HCV, HBV, CMV infection patients confirmed by PCR, and the sera taken from the *S. aureus* septicemia patient. The elimination of the inhibitors were tested of the hemoglobin, BSA, leukocyte DNA and the lamuvidine. The stability of the kit was

tested during 12 months with interval 4 months on the plasma samples pooled HIVRNA. The clinical trial was done on real plasma sample collected from HIV infected and non infected patients and the results were analyzed versus the results get from HIV1 amplicor.
Results and discussions: The process named HIV1 RT-TQPCR to detect and quantify of the HIV1RNA based on the RT real-time PCR technology using the primers and taqman probe specific the gag gene on the HIV1 genome was prepared. Since the process supplied the control [+] and the standards under the RNA, the users could analyzed the real sensitivity of the testing and the test kit as well as the real quantification graph coming from the RNA not the cDNA. The internal control was also supplied under the RNA using the sample primers of the target but the detection probe was difference by the sequence and the fluorophore and this design can help to user monitor the inhibitors in the sample with HIV1RNA detection negative. The evaluation results demonstrated that the limit of detection of the process is 60 copies/ml; the range of the quantification was 10^2-10^8 copies/ml; the reproductivity was high with the quantification from 100 copies/ml; the stability of the kit was at least 12 months; no cross reaction with HBV, HCV, CMV, and *S. aureus*; the inhibitors was eliminated by testing with the kit; and the process gave the same results as the FDA approved for IVD kit, HIV1 Amplicor, when testing on the real samples HIV [+] and HIV [-] with sensitivity and specificity 100% if the Amplicor was considered as gold standards.
Conclusion: Based on the results of the research we have complete the process with the necessary ingredients for a real-time PCR tests as the RNA extraction kit and the cDNA synthesis kit, amplification, positive control and internal control. At the time of the evaluation of HIV1 RT-TQPCR tests, we obtained the following parameters: (1) sensitivity: 100% (2) specificity: 100% (3) threshold detection: 60 copies/ml plasma (4) the amount is about 10^2-10^8 copies/ml (5) durability in storage for at least 12 months (6) is resistant to inhibition. However, in order to be approved as the IVD kit, the more funding will be needed to try the kit in the laboratories ISO 15189 certified for testing of HIV1RNA detection and quantification for clinical applications.

P1-CM03

Resistance of *Escherichia coli* and *Klebsiella pneumoniae* strains isolated in the First central hospital, Mongolia

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Escherichia coli and *Klebsiella pneumoniae* are the most frequent cause of community and hospital-acquired infections, such as urinary tract, intra-abdominal, and skin and soft tissue infections. Until now, little has been known about the incidence and characteristics of *Escherichia coli* or *Klebsiella* strains in Mongolia. This study was performed at the First Central Hospital, a 560-bed tertiary care hospital in Ulaanbaatar city, Mongolia. *E. coli* and *K. pneumoniae* strains were representing both community and hospital-acquired infections, isolated from January 2014 to December 2014. Automated microbiological system (VITEK 2) was used for identification and antimicrobial susceptibilities. Data from consecutive non-duplicate 2549 *E. coli* and 228 *K. pneumoniae* strains were collected of which 70% and 58.5% were susceptible to 3rd generation cephalosporins (3GC), respectively. Resistance rates of trimethoprim/sulfamethoxazole, Amoxicillin/Clavulanic acid, fluoroquinolone, cefazolin, and amikacin were 69% (349/499), 63.6% (253/398), 42.6% (720/1691), 32.2% (814/2530), and 2.8% (40/145), respectively.

E. coli and *K. pneumoniae* were present in 62% (1932/2978), 30% (482/1631), and 51% (47/91) of urinary tract infection (UTI)s, wound, and intra-abdominal infections, respectively. The most common infections were UTIs (70%), followed by wound (18.0%), intra-abdominal infections (2%), and primary bacteremia (1%) infections.35 patients with *E. coli* bacteremia, and 4 patients with *K. pneumoniae* bacteremia, whose clinical data were available, were included in the analysis.
Therefore, continuous antimicrobial susceptibility surveillance is advisable to track emerging resistance in *Enterobacteriaceae* and national guidelines would be tailored accordingly.

P1-CM04

Assessment of laboratory accuracy measures of the GeneXpert system for the detection of *Mycobacterium tuberculosis* (Mtb) in a Philippine tertiary hospital from October 2014 to February 2015

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A study was conducted to determine the accuracy measures (Sensitivity, Specificity, Positive and Negative Predictive Values and Diagnostic Likelihood Ratios) of the automated Nucleic Acid Amplification (NAA) – based TB detection System, GeneXpert™ (Cepheid, CA, USA). The Mtb culture-based automated system BACTEC™ MGIT™ (BD Diagnostics, MD, USA) was used as reference for the accuracy measures. One hundred forty (140) clinical samples (sputum, tracheal aspirates etc.) were submitted for routine analysis from October 2014 to February 2015.
We report the following Detection Accuracy Measures for the GeneXpert system: Sensitivity: 74.2% (95% CI 59.8-89.6%); Specificity 87.1% (95% CI 80.9-93.4%); Positive Predictive Value (PPV): 62.2% (95% CI 46.5-77.8%); and Negative Predictive Value (NPV): 92.2% (95% CI 87.1-97.4%); Positive Diagnostic Likelihood Ratio (PDLR) of 5.8 (95% CI 3.4-9.8) and Negative Diagnostic Likelihood Ratio (NDLR): 0.3 (95% CI 0.2-0.5). A meta-analysis study by Walusimbi *et al*, (2013) estimated sensitivity and Specificity of GeneXpert™ for Mtb 68% and 98% respectively.
The Nucleic Acid Amplification systems like the GeneXpert™ had turn-around times 0.5-4 hours while culture-based systems for Mtb 5-22 days. The value of these accuracy measures promotes the use rapid molecular techniques in addition to conventional culture methods in detecting Mtb especially in the developing countries like the Philippines which experiences high MTb occurrences.
Keywords: GeneXpert™, BACTEC™ MGIT™, Mycobacterium tuberculosis (Mtb), Accuracy measures

P1-CM05

Comparison of the usefulness of arbekacin and vancomycin in treating chronic suppurative otitis media due to methicillin resistant *staphylococcus aureus*

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Background: Methicillin-resistant *Staphylococcus Aureus* (MRSA) is a major cause of ear infections, in which the use of an appropriate

antibiotic is highly important. Accordingly, we attempted to evaluate the clinical usefulness of arbekacin in treating chronic suppurative otitis media (CSOM) by comparing its clinical efficacy and toxicity with those of vancomycin.
Methods: Patient demographics and clinical characteristics according to the most recently published strengthening the reporting of observational studies in epidemiology (STROBE) statement checklist were collected from electronic medical records. Efficacy was classified according to bacterial elimination (BE) or bacteriologic failure (BF) and improved or failed clinical efficacy response (CER).
Results: Ninety-five subjects were diagnosed with MRSA CSOM. Twenty of these subjects were treated with arbekacin, and 36 received treatment with vancomycin. The bacteriological efficacy response (bacterial elimination, treatment with arbekacin vs. vancomycin: 85.0% vs. 97.2%) and clinical efficacy response (improved, treatment with arbekacin vs. vancomycin; 90.0% vs. 97.2%) were not statistically different between the two groups. However, the rate of complications was significantly higher in the vancomycin group (33.3%) than in the arbekacin group (5.0%) (p=0.020). Twelve adverse reactions were observed in the vancomycin group, including two instances of hepatotoxicity, one of nephrotoxicity, eight leukopenias, two skin rashes, and one case of drug fever.
Conclusions: Our data suggested that arbekacin may be a good alternative drug to vancomycin for the treatment of MRSA CSOM.

P1-CM06
Characterization of *Propionibacterium acnes* by multilocus sequence typing and repetitive-sequence-based PCR

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Background: *Propionibacterium acnes* (*P. acnes*) is a Gram-positive and aerotolerant anaerobic bacterium that mostly resides in human skin and plays a critical role in the inflammatory acne. There are limited scientific evidences and reports about molecular epidemiological study of *P. acnes* in Korea as well as in the world.
Methods: A total of 22 *P. acnes* isolates which were originated from diverse patients were obtained from three National Culture Collections for Pathogens (NCCP). Hemolysis was observed on blood agar plates and minimum inhibitory concentrations (MIC) of five antibiotics (tetracycline, doxycycline, clindamycin, erythromycin, and minocycline) were determined using corresponding E-test strips. Multilocus sequence typing (MLST) was analyzed by sequencing eight loci (*aroE, guaA, tly, camp2, atpD, gmk, lepA*, and *sodA*) and applying the expanded *P. acnes* MLST scheme (http://pubmlst.org/pacnes/). Purified genomic DNAs were amplified using the DiversiLab Propionibacterium Fingerprinting kit and the amplified DNA fragments were analyzed using the automated microbial genotyping system.
Results: Among the total isolates, only one isolate showed high MIC values and resistance to all five antibiotics (tetracycline: 8 μg/ml, doxycycline: 4, clindamycin: ≥ 256, erythromycin: ≥ 256, and minocycline: 3). Ten isolates (45%) showed hemolytic properties. MLST results revealed four phylogroups that were typeIA1 (27.3%), typeIA2 (18.2%), typeIB (13.6%), and typeII (40.9%). Repetitive-sequence based PCR results showed two fingerprint types that were T1 (19/22, 86.4%) and T2 (3/22, 13.6%). Three isolates showing T2 types were all typeIB and hemolytic. There was no correlation between MLST results and hemolytic properties.
Conclusion: This study is the first report regarding the molecular epidemiological characteristics of *P. acnes* isolates in Korea using MLST and Repetitive-Sequence-Based PCR. This is expected to have important therapeutic and analytical implications in *P. acnes* burden.

P1-CM07
Real-time PCR for diagnosis of acute respiratory infection: all-in-one solution

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Introduction: Currently only cultivable bacteria causing respiratory infections are detected by traditional culturing techniques. Essentially, detection sensitivity is heavily dependent on differential culture media of which many laboratories would lack; as well as on the transportation duration; on any medications the patients may undertake. Not only that, detection specificity is dependent greatly on sample reliable, which requires technicians to perform macroscopic examination as well as microscopic Gram stain. The remaining of an iceberg, all other uncultivable causative micro-organism such as atypical bacteria or viruses, is undetectable with our current diagnostic methods. In some hospitals, immuno-assay are used to detect specific antibody in sera or specific antigen in specimens. Nevertheless, such methods are not clinically relevant due to its low sensitivity, when detecting antigen, or to cumbersome procedure because of the requirement of the paired sera collected 2 times in 10 days, when detecting antibody. At the moment, many clinical laboratories are equipped with real-time PCR or PCR upgradable to real-time PCR. Therefore, it is totally feasible to utilize these facilities in comprehensive qualitative/quantitative detection of all causative agents including bacteria, atypical bacteria and viruses from patient sample for diagnosis of pathogens causing acute respiratory infections. The principles of real-time PCR is to amplify, detect and quantify specific nucleic acid sequences present in patient samples. This method offers the highest specificity and sensitivity. Moreover, quantitative results will also inform physicians about the status of detected causative agents, whether it is pathogens, or colonized/normal flora.
Materials and methods: For the past years, high-tech microbiological unit at Nguyen Tri Phuong hospital has implemented real-time PCR to detect and quantitate microbial agents in the sputum samples collected from hospitalized patients with diagnosis of acute respiratory infection. Briefly, the method were carried-out as the following: homogenized samples by NALC phosphate buffer (PBS) followed by proceeding 200 μl homogenized sputum to nucleic acid (DNA and RNA) extraction by [NK]MAGBEAD using silica coated bead and KingFisher Flex automated nucleic acid extraction machine; after that, 5 μl extracted nucleic acid solution is added into real-time PCR mix (to detect DNA) or into real-time RT-PCR one-step mix (to detect RNA) which contain required primers and taqman probe specific for the causative agent detection. There are 18 detectable causative agents by this method: 3 prominent community bacterial agents *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*; 2 less-prominent community bacterial agents *S. pyogenes* và *S. agalactiae*; 6 atypical bacteria agents *M. pneumoniae*, *C. pneumoniae*, *L. pneumophila*, *B. pertussis*, *B. parapertussis*, *C. psittacii*; and 8 viral agents *Influenzavirus A*, *Influenzavirus B*, *Parainfluenzavirus* 1, 2, 3, *Respiratory syncytial virus*, *Human metapneumovirus*, and *Adenovirus.* All probes and primers were published in good-impact international journals. In order to ensure the quality of both samples and extracted nucleic acid, in-built real-time PCR to detect house-keeping human gene beta-globulin was used. Other controls include positive controls [+], which is in-house constructed plasmid that contain specific target nucleic acid sequences, and negative controls [-], which is 1X

TE buffer. These controls are to detect any plausible contamination as well as the sensitivity of these PCR mixes. A positive diagnostic result will have a positive threshold cycle number (Ct) above amplification baseline. This Ct value will be use to determine the presenting nucleic acid quantity within 1mL sample volume.

Results: From 1/2014 to 4/2014, there were 124 phlegm samples collected from 124 admitted patients diagnosed for acute respiratory infection at Nguyen Tri Phuong hospital. Real-time PCR and real-time RT-PCR results revealed 75.8% (94/124) samples detected with causative agents. Among these detected samples, further analyses unfolded 46.6% cases positive for community respiratory infection (most prevalently *S. pneumoniae*, followed by *H. influenzae*, *M. catarrhalis*, *S. pyogenes*, and to the least was *S. agalactiae*); 51.6% cases positive for atypical bacterial agents (majority was *M. pneumoniae*, followed by *C. pneumoniae*, *C. psittacii*, Mycoplasma spp., and *B. pertussis*). It is worth to mention that 9.4% cases were detected with viral agents (4 cases detected with *parainfluenzavirus 3*, 3 cases with Influenzavirus A, and the remaining were detected with *Parainfluenzavirus 1*, *2*, *Adenovirus*, *Respiratory syncytial virus*, và *Human metalopneumovirus*). Using the threshold of 10^5 copies/ml, we determined 80 cases (64.5%) with defined causative agents; with the prevalence for each was *S. pneumoniae* (38.75%), *H. influenzae* (13.75%), *M. catarrhalis* (5%), *Mycoplasma spp.* (21.25%), *Chlamydia spp.* (12.5%), *Bordetella pertussis* (3.75%), *Influenzavirus A spp.* (1.25%), *Parainfluenzavirus 1* (1.25%), *Parainfluenzavirus 3* (1.25%) and *Respiratory syncytial virus* (1.25%). There were 32 cases among 80 cases with bacterial agents co-detected with primary causative agents. Further analyses suggested that among 30 cases with atypical bacterial agents: 2 cases (6.67%) were co-infected with *H. influenzae*, 4 cases (13.33%) were co-infected with *S. pneumoniae*, and 1 case (3.33%) was co-infected with *Human metapneumovirus* (3.33%). Among 31 cases where the primary causative agent was *S. pneumoniae*, 10 cases (29.03%) were co-infected with uncommon bacterial agents, 1 case (3.23%) was co-infected with *H. influenzae*, and 2 cases (6.46%) were co-infected with *M. catarrhalis*. Among 11 cases where *H. influenzae* was the primary causative agent, 8 cases were co-infected; 5 cases (45.45%) were co-infected with atypical bacterial agents; 5 cases (45.45%) were co-infected with *S. pneumonia*, and 2 cases (18.18%) were co-infected with viruses. Among 4 cases where primary causative agent were viruses, only 1 case (25%) was co-infected with *S. pneumonia* while none of these cases was co-infected with *M. catarrhalis.*

Discussion: Despite that *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* are reportedly among the most prevalent causative agents for respiratory infection in the world, the current detection capability at Vietnamese hospital clinical laboratories remains very low. This discrepancy is not due to geographic boundary or differences in weather and social-economic status but mainly due to the fact that most hospitals do not have adequate supply of different culture media specifically for these "common but difficult to culture" bacterial agents. Furthermore, sputum sample collection is difficult due to prone to contamination. It is also crucial to process these samples immediately for most accurate results. Yet these two requirements are infeasible to achieve within a clinical laboratory or clinic. For atypical bacterial agents, most laboratories would ignore these agents or only perform ELISA without antibody kinetic test. Hence, there are two common outcomes: either high positive rate (due to most patients would have background antibody), or decline request to perform testing for atypical bacterial agents. With regards to detecting viral agents, nearly none of the current laboratories would perform any diagnostic tests, unless they are done under "sponsorship". Therefore, implementation of real-time PCR and RT real-time PCR has become essentially crucial and appropriate methods of detection. This is because they surpass the differential media type requirements; sample collection requirements; and cumbersome ELISA methods. Importantly, molecular biology approach in diagnostic is totally feasible. This is because many laboratories have been equipped with real-time PCR facility. Even if a laboratory lacks of such facility, the equipment cost is less than 50,000USD (less than an automatic biochemical diagnostic machine). The results of this study also showed that the prevalence of community; atypical bacteria and viral agents among adult patients in Vietnam with acute respiratory infection are now correlated with prevalence elsewhere, which reported high prevalence of *S. pneumoniae*, followed by *Mycoplasma spp.*, *H. influenzae*, *Clamydia spp.*, *M. catarrhalis* and viruses. Our results also suggested co-infection among cases with respiratory infection. Therefore, initial antibiotics choice should be determined in consideration to co-infections, especially those effective on atypical bacterial agents. This is because in our study, 37.5% cases were infected with atypical bacterial agents and among all cases infected with community bacterial agents, 30.43% were also co-infected with atypical bacterial agents.

Conclusion: Applying real-time PCR and RT real-time PCR to detect acute respiratory infection causative agents is the most feasible solution. The feasibility is determined by many folds. Firstly, these methods are the most appropriate to our current hospital conditions. Their simple procedures achieve the highest sensitivity while avoiding difficult requirements of other traditional microbiological methods. These methods also fit the current diagnostic trend worldwide: applied molecular biological techniques for microbiological diagnosis. The methods described in this study only detect 18 communal microbial agents. Nonetheless, it is an open system which detection of other hospital-acquired infectious agents such as *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumanni*... is totally possible. Consequently, we call this technique an "all-in-one solution".

P1-CM08

H. pylori in Vietnam: the complete solution for the clinical laboratory

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Background: Viet Nam with the existing socio-economic conditions of the developing countries, Gastro-duodenal ulcer is one of the most common diseases, but the complete solution for the clinical laboratory targeted the *H. pylori* pathogen that can give more clinical relevant results to the doctor has not been established.

Aims of the study: Carry out a study to detect the *H. pylori* from the gastro-duodenal biopsies, the genotypes of the virulent genes, the antibiotic resistance to the current choice antibiotics, and the CYP2C19 mutations related to the PPI metabolism.

Materials and methods: The studied samples were the biopsies collected from patients with gastro-duodenal ulcer that were sent to the NK-Biotek laboratory for *H. pylori* detection. Briefly, the samples were cultured on the specific selective media for *H. pylori* isolation; then the isolates were carried out the antibiotic susceptibility testing by standard antibiotic agar dilution method with 5 current choice antibiotics. Besides that, the cagA and the s1/s2/m1/m2 allele of the vacA gene of the *H. pylori* were also directly detected in the biopsies by real-time PCR technologies. From the biopsies, the real-time PCR for detection of the 23s DNA_{RNA} mutation related to the clarithromycin were also done and compared to the results of the sensitivity testing. In order to help the clinicians to select the right dose for the PPI, the CYP2C19

mutations detection were also carried out directly from the same samples by real-time PCR.

Results: Compare the PCR versus the culture for detection of the *H. pylori* directly from the biopsies sample, among 433 biopsies sample that were carried out the PCR and the culture simultaneously, 98% were positive by multiplex PCR detecting cagA and vacA gene, and 97% were positive by culture. This results demonstrated that culture give the same sensitivity as PCR for *H. pylori* detection. During the studied period, 765 H. pylori isolates in which 668 from the suspected treatment failure and 97 from the new infected patients were carried out the antibiotic susceptibility testing. Among the suspected treatment failure, 13.6% were resistant to amoxicllin, 49.6% to metronidazol, 62.7% to clarithromycin, 33.4% to levofloxacin, and 18% to tetracycline. Among the new infected isolates, 10.3% were resistant to amoxicllin, 42.3% to metronidazol, 16.5% to clarithromycin, 21.6% to levofloxacin, and 38.1% to tetracycline. These results demonstrated the resistance ratio to clarithromycin among the new infected isolated is dramatically lower than the suspected treatment failure isolates. Seventy three biopsy samples in which 43 resistant and 30 sensible to clarithromycin, confirmed by susceptibility testing of clarithromycin of the isolates, were carried out the A2142G/C, A2143G, and T2182C mutation detection on the domain V of the 23s. The received results revealed that no A2142G/C mutation were detected among both resistant and sensible isolates; 100% of the resistant isolates carried the A2143G, but the sensible isolates also carried this mutation with very high ratio (90%). About the T2182C mutation, both the resistant isolates and the sensible isolates carried this mutation with very high ratio: 95% and 97% respectively. These findings demonstrated that no mutation for high level resistance to clarithromycin (A2142G/C) were detected among the studied isolates, and the role of the mutation at position 2143 and 2182 of the 23s gene must be further investigated through the correlation of those mutation with the chlarithromycin MIC. The distribution of the cagA gen and the different genotype vacA detected from *H. pylori* originated from children (39) adult (168) were also received and analyzed. The received results said that 44% from children carried cagA gene versus 65% from adult. The distribution of the cagA and vacA genotypes among the children were: cagA[+]s1m1 (31%), cagA[+]s1m2 (13%), cagA[+]s2m1 (0%), cagA[+]s2m2 (0%), cagA[-]s1m1 (13%), cagA[-]s1m2 (44%), cagA[-] s2m1 (0%), cagA[-] s2m2 (0%); and the distribution of the cagA and vacA genotype among the adult are: cagA[+]s1m1 (36%), cagA[+]s1m2 (25%), cagA[+]s2m1 (4%), cagA[+]s2m2 (1%), cagA[-]s1m1 (10%), cagA[-] s1m2 (23%), cagA[-]s2m1 (0%), cagA[-] s2m2 (1%). The obtained results also said that in children, the cagA [+] carried high ratio (71%) of virulent genotype of vacA gene (s1m1) than in adult (55%). Among 714 patients that the CYP2C19 mutation detection were carried-out, surprisingly that 44.8% were among the extensive metabolism, 44.6% among the intermediate metabolism and only 8.5% were among the poor metabolism. This finding demonstrated that the medical doctor should investigate the CYP2C19 mutation in the patient in order to prescribe the suitable personal dose of PPI for achieving the highest effect.

Conclusion: The gold standard to confirm the diagnostic of gastro-duodenal ulcer causing by *H. pylori* is the detection this pathogen from the biopsy taken from the patient's lesion. CLO test that are currently applied in the hospital can quickly reply to the doctor the existence of the *H. pylori* in the biopsy. However, CLO test could not supplied enough essential information to help the doctor to give the most effective treatment regime to the patients. The lab tests including: (1) culture and antibiotic susceptibility testing of the *H. pylori* isolate, (2) direct detection and identify of the virulent genotype of H. pylori existing in the biopsy sample, (3) direct detection of the 23s mutation related to clarithromycin, and (4) detection the CYP2C19 mutation related to PPI metabolism is the complete solution that can be applied in the clinical laboratory since the suitable media for culture of H. pylori are available and the real-time PCR technology can be set-up easily with the affordable funding.

P1-CM09

Correlation of quantitative HBsAg with HBV DNA levels in Malaysian patients with chronic Hepatitis B infection

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Quantitating HBV DNA level by molecular-method is considered expensive particularly in Asia, thus a cheaper HBsAg quantification as a surrogate marker is crucial to reduce the cost in the management of chronic hepatitis B infection (CHB). The correlation between quantitative HBsAg and HBV DNA level will be determined in this study. A total of 162 CHB patients who had undergone HBV DNA by real time polymerase chain reaction were enrolled from University Malaya Medical Centre between June 2009 to June 2011. Patients were categorized based on disease status (CHB without complications; CHB with complications), HBeAg status (positive; negative) and treatment status (with treatment; without treatment). HBsAg was quantified by chemiluminescent microparticle immunoassay. The correlation was analysed using the Spearman rank correlation test(r value). The overall correlation between quantitative HBsAg and HBV DNA level was significant($r=0.293$, $p<0.001$). A significant correlation were observed in the HBeAg positive ($r=0.266$, $p=0.049$) alone and those without complications ($r=0.346$, $p=0.029$). Correlation was also observed in patients without antiviral treatment ($r=0.355$, $p=0.01$). HBsAg showed significant correlation with HBV DNA especially in HBe antigen-positive patient without complications and in the treatment-naïve group.

P1-CM10

Panel strain construction of *Klebsiella pneumoniae* and its phenotypic and genotypic characterization

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Background: *Klebsiella pnemoniae* has been responsible for the infections caused in hospitals, causing mortality and morbidity. There have been constant efforts carried out to control the infections using antibiotic therapy. *Klebsiella pneumoniae* has been evolving as a Multi-drug resistant pathogen, acquiring multiple resistance such as horizontal gene transfer, transposon mediated insertions or change in outer membrane permeability. Considering the severity of the acquired resistance, we have developed a panel of strains of *Klebsiella pnemoniae* expressing different resistance such as high level penicillinase and AmpC production, extended spectrum beta lactamases and carbapenemase producing strains.

Methods: All the bacterial strains were collected during the period 2008-2013 from Severance hospital, Seoul. Strains expressing different resistance were selected according to the phenotypic detection scheme as mentioned in Courvalin, P., et al. Antibiogram ASM press, 2010. In vitro antimicrobial susceptibility was determined by CLSI agar dilution method. Selected strains were sequenced using Ion Torrent PGM system (Life Technologies,

USA). Annotation and analysis were performed using RAST annotation pipeline (http://rast.nmpdr.org/) and Geneious pro 8.0 (Biomatters, New Zealand) respectively.
Results: Bacterial strains expressing different resistance phenotypes were collected and genotypically characterized. Whole genome sequences of the resistant strains were examined for the resistance gene, mutations and porin alterations contributing to the detected phenotypes. We encountered several discrepancies in the traditional phenotypic scheme while selecting the strains with specific resistance. In depth analysis of the whole genome sequence indicated the insertions of multiple resistance genes and factors offering the intrinsic resistance.
Conclusion: Rapid development within the field of Massive parallel sequencing (MPS) has enabled us to get a better insight regarding the bacterial resistance in the clinical microbiology laboratory. Using the above technology we have constructed the panel strains expressing different resistance in *Klebsiella pneumoniae*. These Panel strains can be used in the clinical laboratory as the reference strains. In addition, these strains could to be significant in the field of pharmaceuticals for the antibiotic drug testing.

P1-CM11
Characteristics of *bft* genes among enterotoxigenic *Bacteroides fragilis* isolates from extraintestinal specimens
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Background: Enterotoxigenic *Bacteroides fragilis* (ETBF) produces enterotoxin regarded as a virulence factor. Three different isotypes of *B. fragilis* enterotoxin (*bft*) gene have been identified as *bft-1*, *bft-2* and *bft-3*. In this study, the presence of ETBF in clinical *B. fragilis* isolates were investigated and the antimicrobial resistance of *bft*-positive and -negative isolates were determined.
Methods: A total of 537 *B. fragilis* isolates were collected from extraintestinal specimens between 2006 and 2013 at a university hospital in Korea. Multiplex PCR was applied to detect *bft* gene isotypes. Antimicrobial susceptibilities of 107 *B. fragilis* isolates including 33 *bft* positive and 74 *bft* negative were determined by the CLSI agar dilution method.
Results: Proportion of ETBF was 30% (162 of 537) of *B. fragilis* isolates, 33% (48 of 144) in blood isolates and 29% (114 of 393) in other extraintestinal isolates. Among the ETBF isolates, *bft-1* was the most common isotype (77%), followed by *bft-2* (14%) and *bft-3* (9%). The antimicrobial resistance rates (%) of *bft*-positive and -negative isolates were piperacillin (33 vs. 24), piperacillin-tazobactam (3 vs. 2.7), cefoxitin (6 vs. 5), cefotetan (15 vs. 10), imipenem (3 vs. 1), clindamycin (42 vs. 38), and tigecycline (21 vs. 18), whereas all tested isolates were susceptible to chloramphenicol and metronidazole.
Conclusions: The proportion of ETBF in blood isolates is similar to that of other extraintestinal isolates and *bft-1* is the predominant isotype. The resistance rates of *bft*-positive isolates to antimicrobial agents are observed to be higher than *bft*-negative isolates, although the difference is not statistically significant.

P1-CM13
Clinical characteristics and risk factors for hand-foot-mouth disease with severe neurologic complications in South Korea, 2009-2014
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Introduction: A nationwide sentinel surveillance system for HFMD with neurologic complications was initiated in 2009. In this study, we investigated clinical characteristics and risk factors of HFMD with severe neurologic complications using the system.
Methods: A retrospective review of medical records was conducted on all cases reported to this system from 1 April 2009 until 31 December 2014. Severe case was defined as having HFMD or herpangina (HP) with encephalitis or polio-like syndrome or cardiopulmonary complications, and less-severe case was defined as having HFMD or HP with aseptic meningitis .
Results: A total of 138 severe (34.8%, 48/138) or less-severe (65.2%, 90/138) cases were included from 28 hospitals, excluding inappropriate 28 cases from 166. Of those 48 severe cases, 27 (56.2%, 27/48) showed encephalitis: 14 (29.2%, 14/48), polio-like syndrome: and 7 (14.6%, 17/48), cardiopulmonary syndrome. Median age was 36 months (range 1-381, 2Q 18, 4Q 60) and 75 (54.3%) were male-gender. The patients were completely recovered except 7 fatal cases (5.1%, 7/138; neurologic sequele 3, death 4) Comparing clinical variables between severe and less-severe diseases by multivariate analysis, lethargy (OR 5.527 p=0.039 95% CI 1.09-28.13), absence of neck stiffness (OR 0.77, p=0.009, 95% CI 0.01-0.53), higher ESR (OR 1.04, p=0.045, 95% CI 1.00-1.08) and lower blood phosphate level (OR 0.22, p=0.005, 95% CI 0.08-0.63) were significantly associated with HFMD with severe neurologic complications.
Conclusion: Presence of lethargy, absence of neck stiffness, higher ESR and lower blood phosphate level in patients with HFMD may indicate severe neurologic complications.

P1-CM14
The usefulness of the Widal agglutination test in diagnosis of enteric fever in Sri Lanka
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Background: While the definitive diagnosis of enteric fever requires isolation of Salmonella Typhi or Paratyphi from blood, serological diagnosis using the Widal test is widely practiced. The high cost, long turnaround time and non-availability of cultures have compelled the clinicians to use the serology which is cheap, rapid, and widely available. Looking for four fold rise in titres though recommended is not practiced due to the non-practicality. About 7500 and 2000 Widal agglutination tests are performed annually in five main private hospitals and in main government hospitals in Colombo district. The usefulness of the test should be evaluated in our country as there had been several instances where antibiotics have been prescribed irrationally, based on low titres and ambiguous results of the test.
The present study was carried out to determine the usefulness of a single value of the Widal test to diagnose enteric fever in Sri Lanka
Method: 325 healthy volunteers representing all provinces of Sri Lanka, according to the percentage of population in 2010 statistics, 56 patients diagnosed with enteric fever with a blood culture

positive for *Salmonella Typhi* or *Paratyphi A* and 180 patients who had febrile illness other than enteric fever diagnosed clinically with the supportive investigations were included in this study.
The information was collected using an interviewer administered questionnaire and from the patient notes.
Widal tube titration test was performed according to the procedure described in the Mackie and McCartney Practical medical microbiology 14th edition. Bacterial antigen in-house preparation was purchased from the Medical Research Institute, Borella, Sri Lanka.
Results: The healthy volunteers showed higher sero prevalence to O antigen than to H and AH antigen. There was a detectable geographical variation of sero prevalence to these antigens. Non-enteric fever febrile group demonstrated a significant cross reaction in H and AH antibodies. The chosen cut off was1:320. At this titre, O, H and AH antibodies had low sensitivity and high specificity. The O antibody was the better indicator than H antibody at this titre for infections with *Salmonella Typhi.* The Widal test showed a good negative predictive value for all three antibodies.
Conclusions: ≥1/320 titre in O, H and AH antibodies in the Widal test could be helpful in making a diagnosis of enteric fever in an appropriate clinical setting. A negative test can be used to exclude enteric fever in a febrile patient.

P1-CM15
Pilot screening of antibiotics synergy using the characterized multidrug resistant bacterial strain library
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Background: As multidrug-resistant (MDR) bacteria recently increase in the clinical setting, it is important to seek new antimicrobials or the new strategy for avoiding the multidrug resistance. As part of avoiding, we performed the pilot study for screening the combination of carbapenem and other antimicrobials for MDR gram negative bacteria or vancomycin and other antibiotics for MDR gram positive bacteria.
Methods: Forty five of strains were used in the strain library including 23 of carbapenem resistant gram negative strains, 16 vancymicin resistant or intermediate gram positive and 6 control strain. Total fractional inhibitory concentrations (FIC) of imipenem (IMP) or vancomycin (VAN) combined with ciproproxacin (CIP), gentamycin (GEN), and trimethoprim-sulfamethoxazole (TMP/SMX) was measured in the checkerboard pattern of a broth microdilution assay.
Results: In the combination of IMP plus TMP/SMX, 87.5% of carbapenem resistant *Acinetocater baumannii* and *Escherichia coli* strains exhibited synergy ($\sum$ FIC values ≤ 0.5) and partial synergy (0.5 < $\sum$ FIC values ≤ 1). In *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains, the combination of IMP plus CIP was more effective (synergyand partial synergy= 80%). In *Enterococcus faecium* and *Staphylococcus aureus*, the combination of IMP plus CIP was more effective (71.4% and 55.6%, respectively).
Conclusion: The different combinational effects for synergy among species were shown. These results suggest that the screening of synergy could be useful for providing clues for selecting the combination strategy and the treatment of MDR strains. Further studies should be needed for the therapy combined with the next generation or others extensively.

P1-CM16
Rapid detection of β-lactam antibiotic resistance in bacteria by instrumental analysis
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β-lactam antibiotics are most widely used but the outcome of antimicrobial resistance (AMR) is one of the most serious public health threats. The induction of β-lactamase is main mechanism of AMR. The resistance mostly involves the chemical modification of an antimicrobials to an inactive form by the released enzyme from bacteria. The modification causes structure change and is followed by the characteristic mass shift of the antimicrobials. Using this mechanism, we developed new liquid chromatography-mass spectrometry based on quantitative analysis to determine the amount of resistance to penicillin G, ampicillin and amoxicillin in *Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium,* respectively. This method was successfully applied to 48 bacterial isolates from the Korean farm. The duration to test their resistance took only for 2 hours. This developed method may contribute to quantitatively and qualitatively analyze the amount of AMR and can compare their correlation with traditionally microbiological parameter such as MIC (Minimum Inhibitory Concentration).

P1-CM17
Comparative analysis of LipL32 genes in *Leptospira spp.* from human and environmental sources
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Lipoproteins LipL32 is renowned as a highly conserved major part of the outer membrane and is one of the virulence factors for pathogenic *Leptospira* spp. In this study, we investigated whether leptospires from environmental water sources possess LipL32 gene similar to that of the human leptospires. A total of 60 DNA of *Leptospira* spp., comprising 30 from the environment and 30 from human that were PCR-positive for LipL32, was sequenced. It was found that the nucleotide sequences of LipL32 of all the leptospires from the environment were homologous with 99.9% similarity to the LipL32 from human leptospires. Thus, it can be concluded that LipL32 is a very conserved gene in both human and environment leptospires, but its role as a virulence factor in environmental leptospires is yet to be confirmed.

P1-CM18
Surveillance of bloodstream pathogens and their antibiotic resistance in a Cambodian referral hospital (2007-2013)
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Background: Bloodstream infections (BSI) cause important morbidity and mortality worldwide, and especially in low resources settings. We describe the results of a prospective blood culture based surveillance study in an adults' hospital in Cambodia (2007-2013).
Methods: Blood cultures were performed for all adult patients presenting with systemic inflammatory response syndrome

(SIRS) at Sihanouk Hospital Centre of HOPE, Phnom Penh, Cambodia. Isolates were identified using standard microbiological techniques; antibiotic susceptibilities were assessed using disk diffusion and MicroScan, with additional E-test, D-test and double disk test, according to CLSI guidelines.
Results: A total of 14291 samples from 10352 patients yielded 1248 clinically significant organisms (8.7%). The most frequent pathogens included *Escherichia coli* (n = 365; 29.2%); *Salmonella* spp. (n = 214, 17.3%); *Burkholderia pseudomallei* (n = 119; 9.6%), *Klebsiella* spp. (n = 86; 7.0%); and *Staphylococcus aureus* (n = 110, 8.8%). Methicillin resistance was seen in 28/110 (26%) of *S. aureus*. Among *E. coli,* we noted combined resistance to amoxicillin, SMX-TMP and ciprofloxacin in 226 isolates (62%) and 55% (137/248 isolates tested) were confirmed as producers of extended spectrum beta-lactamase (ESBL). For *Salmonella* Typhi (and other *Salmonella* spp.), there is a trend of increasing reduced susceptibility to ciprofloxacin but resistance to 3rd generation cephalosporins was less common. Two patients presented with an invasive carbapenem resistant *Klebsiella pneumoniae* infection. For *Burkholderia pseudomallei,* no resistance towards the drugs of use (i.e. ceftazidime, cotrimoxazole, amoxicillin-clavulanic acid) was noted.
Conclusions: BSI in Cambodian adults is mainly caused by difficult-to-treat pathogens. These data urge for sustained surveillance through microbiological capacity building, and solid interventions to contain antibiotic resistance.

P1-CM19
High Panton-Valentine leukocidin (PVL)-positive methicillin sensitive *Staphylococcus aureus* (MSSA) strains among HIV patients in Sungai Buloh Hospital, Malaysia
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Background: Panton-Valentine leukocidin (PVL) is a toxin produced by less than 2% of *S. aureus* which usually presents as skin and soft tissue infections (SSTI) such as cellulitis, recurrent boils and carbuncles and rarely causes invasive infections such as necrotizing pneumonitis. Infections caused by this strain can be debilitating and additional antibiotics are used as an adjunct to treat this condition. Cases of PVL positive *S.aureus* are increasing among HIV patients and children. Therefore the objective of this study is to determine and investigate the prevalence and the infections caused by PVL positive S. *aureus* strains among HIV positive patients in one of the HIV reference centre in Malaysia.
Methods: A total of 129 HIV positive patients had swabs taken from four different sites of their body parts and screened for the presence of *S. aureus* during HIV clinic follow-up from December 2011 to January 2012. *S. aureus* strains obtained were identified using the conventional method and VITEK 2 was used to determine their sensitivity to methicillin and the minimum inhibitory concentration of the antibiotic. The *S. aureus* strains were then subjected for DNA extraction and polymerase chain reaction (PCR) was carried out to detect the PVL gene.
Results: Results revealed that 39 patients (30%) were colonized with methicillin sensitive *S. aureus* (MSSA) and only one patient was colonized with methicillin resistant strain (MRSA). Of these 40 colonizers, 33 (82.5%) patients were found to harbor PVL positive strains of *S. aureus*. Only three patients had documented SSTI.
Conclusion: Although we found an unusually high PVL positive strain S. *aureus* among our HIV patients, the rates of SSTI are still low. Nevertheless its detection is useful as appropriate measures can be taken to prevent relapse to the patients and also to prevent the spread to others.

P1-CM20
Usefulness of cattle blood as an enrichment substance in blood supplemented culture media, in the clinical microbiology laboratory.
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Background: Sheep blood is considered as one of the best enrichment agents used in preparation of blood supplemented media. In Sri Lanka we use expired banked human blood for preparation of culture media as sheep and horse blood is not freely available. But this has poor haemolysis pattern, possible poor growth due to inhibitors and possible risk of blood born infections. In Sri Lanka the population of cattle is 44 times higher than that of sheep population.
A study done in 2009 in Sri Lanka to compare cattle, human, sheep and rabbit blood using quality control strains showed that haemolysis pattern of cattle blood was almost similar to that of sheep and rabbit blood.
The current study was carried out to determine the ability of cattle blood as an enrichment substance in blood supplemented media in microbiology.
Method: 303 clinical samples were processed during the study period on cattle, human and sheep blood containing medium. Isolation rates, colony appearance, pattern of haemolysis, relevant identification tests and antimicrobial susceptibility were compared qualitatively with the pattern on sheep blood.
Results: Human blood supplemented chocolate agar could isolate 100% while cattle and sheep blood supplemented media could only isolate 75% and 50% of *Haemophilus* species respectively. All the other organisms grew on all the media.
Colony sizes in human blood supplemented blood and chocolate agar is obviously smaller than colony sizes in cattle and sheep blood supplemented blood and chocolate agar. All the Gram Negative bacteria had the same colony size and morphology in all three media.
Zones of haemolysis of Streptococcal species were smaller in human blood supplemented agar but almost equal in cattle and sheep blood supplemented agar.
CAMP test showed positive results for Group B Streptococcus and negative results in negative control with sheep as well as cattle blood but not with human blood supplemented media. Optochin and bacitracin sensitivity and ABST results were similar in all three media.
Conclusions: Human blood supplemented chocolate agar is better than cattle and sheep blood supplemented chocolate agar for isolation of *Haemophilus* species from clinical specimens
Cattle blood supplemented media has almost all qualities of sheep blood agar which are useful in identification of bacteria e.g. Colony morphology, Haemolysis, CAMP test and optochin sensitivity.
ABST done according to CLSI method also gave similar zone diameters with all 3 types of media.

P1-CM21
Genotyping of *Staphylococcus aureus* colonizing HIV infected patients
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Staphylococcus aureus colonized multiple sites on the human body and is most predominant in the anterior nares. Patients with HIV who are *S. aureus* carriers are at most risk of developing bacteraemia and deep soft tissue infections. In this study, our

objective was to determine the clonality of *S. aureus* carriage in 129 HIV patients who attended an outpatient clinic at a tertiary care hospital in Malaysia. Isolates of *S. aureus* were collected from four sites (anterior nares, axilla throat and skin) of the patients. From the total number of patients, 27.1% were positive for *S. aureus* with seven patients having isolates obtained from more than one site. From the total of 43 strains obtained, only one strain was resistant to methicillin. Pulsed field gel electrophoresis performed on all the strains which consisted of 20 nares, 13 throats, 8 skin and 2 axilla revealed that the *S. aureus* strains were grouped into 18 types. It was also found that six strains isolated from three patients who had *S. aureus* obtained from multiple sites were distributed into different groups, whereas four patients had *S. aureus* that were grouped together. There was no clonality of strains from common sites and the MRSA strain was on its own. Although the number of samples are limited, our result showed that *S. aureus* that colonize on the cohort of HIV infections patients are genetically diverse.

P1-CM22

Multiple myeloma as a major cause of false-positive galactomannan tests in adult patients with cancer

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Background: The galactomannan (GM) test is a useful method for early diagnosis of invasive aspergillosis, but various factors can cause false-positive results. Recent products of piperacillin/tazobactam, previously considered a major cause of GM false-positivity, are reported not to be related to it. Meanwhile, multiple myeloma has newly been suggested to be a risk factor. To validate these findings, we performed a case-control study in adult patients with cancer admitted at a tertiary care university hospital.
Methods: A case-control study was designed to evaluate the effects of antibiotic use and underlying malignancies on GM false-positivity. Electronic medical records were reviewed for patients admitted March through June 2014. Patients with false-positive GM results were selected as cases and those with negatives as controls. To verify the results of the four-month analysis, additional analysis was performed in multiple myeloma patients over a three-year period.
Results: There were 30 false-positive and 316 negative cases during the four-month period. Among the factors evaluated, multiple myeloma was the only significant factor in the adjusted analysis (OR = 3.59, CI 1.28-10.04). In the three-year analysis of 145 multiple myeloma patients, 25.5% showed false-positive results, which was 3 times higher than overall. GM false-positivity was not related to serum monoclonal protein level or type of immunoglobulin. GM optical density indexes (ODIs) in all false positives were lower than 3.0.
Conclusions: Multiple myeloma was a major cause of GM false-positivity in adult cancer patients. GM was false-positive in 25.5% of multiple myeloma patients with GM ODIs lower than 3.0.

P1-CM23

The effect of antimicrobial therapy prior to blood culture on the mortality

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Background: Blood should be collected prior to the initiation of antimicrobial therapy for blood culture. However, antimicrobials are already prescribed in many clinical situations. The aim of this study was to analyze the frequency of antimicrobial therapy before blood culture in patients admitted to Gyeongsang National University Hospital (GNUH), and its effect on the mortality.
Methods: The frequency of antibiotic usage before blood culture in each department, the antimicrobial agents used, and mortality were reviewed retrospectively by electronic medical record at GNUH. For blood culture, the BacT/Alert System (bioMerieux) was used with standard bottles (SA and SN). Two sets of blood culture were performed for each episode.
Results: Among 900 patients enrolled, the prescription rate before blood culture was highest in the MICU (90%), SICU (76%), and departments of surgery (92%), hematology (76%), and neurosurgery (62%). The prescription rate before blood culture was 48.0% (432/900). A total of 1,096 prescriptions were written for these patients. Cephalosporins (31%) were most commonly prescribed, followed by penicillins (16%), quinolones (14%), and antifungal agents (9%). Among 432 patients receiving antibiotics before blood culture, 47 (10.9%) showed bacteremia or candidemia. Nine patients were positive for *Staphylococcus aureus*; 8 for *Enterococcus faecium*; 8 for *Escherichia coli*; 6 for *Klebsiella pneumoniae*; and 4 for *Candida albicans*. Among 468 patients who did not receive antibiotics, blood cultures from 36 (7.7%) grew microorganisms, including *S. aureus* in 8 cultures and *E. coli* in 4. The mortality rate of patients testing positive for pathogens who received antibiotics was 36.2% (17/47), which is significantly higher than 11.1% (4/36) in the patients who did not receive antibiotics.
Conclusion: Approximately 50% of the inpatients had received antimicrobial therapy prior to blood culture. Cephalosporins, penicillins, and quinolones were the most common antibiotics prescribed before blood collection. The severity of disease, compromised immunity, suppression of normal flora by antibiotics, and additional underlying illnesses may have played a role in the poor prognosis among the patients who had received antibiotics.

P1-CM24

Frequency of *Listeria monocytogenes* occurrence in 2nd and 3rd trimesters in pregnant women referred to Emam khomeini and Razi hospitals in Ahvaz, Iran

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Background: *Listeria monocytogenes* is a Gram-positive bacterium, found in soil, water, decaying vegetables, and milk products. Listeriosis is transmitted from animals to humans. This bacterium causes an influenza-like illness, or self-limiting enteritis. It may lead to serious disease in the elderly, infants, pregnant women and those who are immune compromised.
Objectives: The purpose of this study was to detect *L. monocytogenes* in vaginal specimens by the culture and PCR methods, and perform a comparison between them.
Patients and Methods: A total of 354 women (mean age 32.3 ± 4.6 years) from January to June , 2013 were included in this study. Vaginal specimens were cultured on PALCAM agar medium and the suspected colonies of *L. monocytogenes* were evaluated by PCR using primers for hly A gene. This gene is specifying the species of *L. monocytogenes.*
Results: Eight samples out of 354 were positive for *L. monocytogenes* by culture (seven in 3rd and one in 2nd trimester). All positive samples confirmed by PCR. Four under study women

who their samples were positive by culture and PCR had abortion in their history (50%).
Conclusions: This fact that the culture and PCR of 50% of women who had abortion became positive confirms the important role of *L. monocytogenes* in the abortion of pregnant women.
Keywords: vaginal specimens; *Listeria monocytogenes*; culture; PCR

P1-CM25
The nasopharyngeal carriage rate, serotype distribution and antimicrobial resistance of *Haemophilus influenzae* among children with upper respiratory infection in Beijing
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Background: Haemophilus influenzae is an important human pathogen that causes severe infections including meningitis, sepsis, and bacteraemic pneumonia, mostly affecting young children. To investigate the nasopharyngeal carriage rate, serotype distribution and antimicrobial resistance of *Haemophilus influenzae* among children visiting an outpatient department in Beijing Children's Hospital with upper respiratory infection from March 2013 to February 2014.
Methods: The serotypes were determined by the latex-agglutination and the antibiotic susceptibility was tested by E-test method.
Results: The nasopharyngeal carriage rate for *Haemophilus influenzae* was 9.2% (271/2930), major in April to June. The number of boys was larger than that of girls', and mostly aged 4~6years old. All of the isolates were non-typable. The susceptibility to tetracycline, chloramphenicol, amoxycillin/clavulanate, and cefuroxime were 97.0%, 96.0%, 91.0% and 82.0%, respectively. The non-susceptibility to penicillin was 35.0%. The carriage rate of beta-lactamase was 23.0%.
Conclusion: About 9.2% of children with upper respiratory infection were nasopharyngeal colonized by *Haemophilus influenzae*. The infection is closely related with age, gender and season in Beijing. The non-susceptibility to penicillin was high, and the beta-lactamase positive rate of *Haemophilus influenzae* was high and increased rapidly.
Keywords: *Haemophilus influenzae*; carriage rate; serotype; antibiotic resistance

P1-CM26
Disseminated *Penicillium Marneffi* infections among HIV patients:correlation of clinical and histology findings
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Penicillium Marneffei (PM) infection is one of the opportunistic infections that can occur in those who are in the late stage of HIV or having AIDS. PM has been isolated from soil and bamboo rat in this part of Asia. In disseminated PM infection, it has a unique clinical feature of papules with central necrosis area which have a distribution on the face, trunks and the extremities. There are also laboratory tests which help in the diagnosis of disseminated PM infections such as serology testing looking for Aspergillus galactomanan antibody and histology examination of skin biopsies. However, to confirm an infection, a positive culture of the fungus itself is still the gold standard. Therefore we performed a study to examine the correlation of skin lesions in HIV patients suspected of disseminated PM infections with histology findings. A total of 20 HIV patients suspected of a disseminated PM infections were referred to the skin clinic in Hospital Sungai Buloh and subjected for skin biopsies. Eighteen of the histology sections showed a typical feature of PM with spherical to oval shaped fungal body with transverse septa. Only 2 did not show obvious transverse septa. Due to the rise in the occurrence of disseminated PM infections among HIV patients and a high correlation between clinical and histological findings, perhaps when there is an increase in the index of suspicion of PM infections in HIV patients, a histological section without culture result is adequate in the diagnosis of PM infections.

P1-ER01
Vancomycin resistant enterococcal (VRE) colonization among patients treated in intensive care units at the national hospital of Sri Lanka (NHSL) and genotype/s responsible for resistance
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Background: VRE have become more prevalent as a cause of nosocomial infection in most parts of the world. The aim of this study was to assess the epidemiology of VRE colonization among patients in the intensive care units (ICU) of the NHSL.
Methods: A cross sectional study was carried out in a total of 218 patients admitted to 12 ICUs of the National Hospital of Sri Lanka from January 2012 to March 2012. Data collected using a questionnaire and by reviewing patient medical records. Rectal swabs were collected on day 0, 4 and 8 and every 4th day thereafter till discharge. Enterococci were isolated from stool samples and identified up to species level using standard bacteriological procedures. Standardized antibiotic susceptibility testing to ampicillin teicoplanin and vancomycin was performed using the Clinical and Laboratory Standards Institute (CLSI) method. Minimum inhibitory concentrations to vancomycin were determined using the E-test in strains showing intermediate or frank resistance to vancomycin. Genotype determination of van A and van B was carried out on isolates identified as VRE using polymerase chain reaction. Patients positive for VRE colonization were followed up to discharge or death.
Results: VRE prevalence in the study sample was 5% (95% confidence interval). Univariate analysis showed that the use of metronidazole (p=0.04) or teicoplanin (p=0.02) or the presence of diabetes (p=0.026) were associated with an increased risk of VRE colonization. Use of cephalosporins or vancomycin were not associated with increased risk (p>0.05).
Conclusion: The 5% prevalence of VRE colonization detected in this study signals the emergence of VRE in the intensive care setting in Sri Lanka. Rational use of antibiotics, such as metronidazole, may be necessary to prevent colonization.

P1-ER02
Antimicrobial susceptibility of VanC-type enterococcal isolates at a university hospital in Korea
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Background: *Eneterococcus casseliflavus* and *Enterococcus gallinarum* (intrinsic VanC-type vancomycin-resistant enterococci) have been associated with human infections much less frequently than *E. faecalis* and *E. faecium*. However, these species may cause serious infections. Few studies have focused on the susceptibilities

of VanC-type enterococci in Korea. The aim of this study was to determine the recent antimicrobial susceptibility of these species at a university hospital in Korea.
Methods: The medical records of patients from 2009 to 2014 at a university hospital in Korea were reviewed retrospectively. Bacterial identification and antimicrobial susceptibility testing were performed by using VITEK 2 system (BioMérieux, Marcy l'Etoile, France).
Results: The number of non-duplicate isolates of *E. casseliflavus* and *E. gallinarum* were 106 and 150, respectively. Among the 256 isolates, 71 (27.7%), 63 (24.6%) and 122 (47.7%) were recovered from bile, blood, and others, respectively. Susceptibility rates of *E. casseliflavus* and *E. gallinarum* were as follows; to penicillin G (98.1% and 87.8%), ampicillin (100% and 93.3%), vancomycin (97.1% and 85.3%), teicoplanin (98.1% and 97.3%), linezolid (99.1% and 100%), erythromycin (42.5% and 69.3%), tetracycline (89.6% and 45.3%), ciprofloxacin (94.3% and 88.0%), nitrofurantoin (100% and 89.2%), high-level gentamicin (99.0% and 90.5%), and high-level streptomycin (96.2% and 82.4%), respectively.
Conclusion: Majority of VanC-type enterococcal isolates are susceptible to antimicrobial agents tested, except to erythromycin and tetracycline. Isolates of *E. casseliflavus* are more often susceptible to antimicrobial agents than *E. gallinarum* except to erythromycin.

P1-ER03
Molecular characterization of vancomycin-resistant *Enterococcus faecium* isolated from newly admitting patients transferred from various regions of Korea
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Background: *Enterococcus* spp. have emerged as important nosocomial pathogens. Especially, vancomycin-resistant *Enterococcus faecium* (VREF) has widely spread in Korean hospitals. In this study, we investigated the molecular characteristics of VREF strains isolated through active surveillance of the newly admitting patients transferred from various regions in South Korea.
Methods: A total of 50 VREF isolates were collected from the rectal swab culture of the newly admitting patients transferred from various regions to Samsung Medical Center in Korea from April to November 2014. Antimicrobial susceptibility testing was determined by VITEK2 (bioMerieux) and PCR was performed to detect vancomycin resistant genes (*vanA/B/C/D/E/G*) and virulence factors (*asa1, gelE, cylA, esp and hyl*). MLST was performed to determine the epidemiological relatedness of the isolates.
Results: All 50 isolates contained *vanA* gene and 32 isolates (64%) had both *esp* (enterococcal surface protein) and *hyl* (hyaluronidase). Most isolates were resistant to ampicillin, clindamycin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, erythromycin, and imipenem, while susceptible to linezolid and tigecycline. The most frequent sequence type (ST) was ST17 (24%), followed by ST230 (10%) and ST78 (6%). The other 22 different STs were represented by one or two isolates. All the strains except one belonged to clonal complex (CC) 17.
Conclusion: Our study shows that most of VREF strains isolated from the newly-admitting patients with variable geographical origin belong to CC17, suggesting the possibility of nationwide transmission of those strains. A substantial proportion of the isolates carry *esp* and *hyl* genes that are well-known virulence factor in CC17 strains. A comprehensive strategy to prevent transmission of VREF between hospitals is necessary.

P1-ER04
Antimicrobial resistance and virulence factors in *Enterococcus* spp. isolated from horses in Korea
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Background: Enterococci are gram-positive cocci that are present as the commensal organism of the intestinal tracts of animals and humans. Their abilities to acquire resistance against antimicrobials and harbor putative virulence traits are considered as main reasons of their opportunistic infections. As horse industry for leisure grows gradually in Korea, the cross-transmission of these pathogen can increase. So the surveillance for *Enterococcus* spp. obtained from horses should be needed.
Methods: A total of 3,078 swab samples were obtained from horses and horse-associated environments in Korea and *Enterococcus* spp. were speciated using specific PCR and VITEK II. After antimicrobial susceptibility tests were performed by using disc diffusion method according to CLSI guideline, presence of the antimicrobial resistance genes and virulence genes were determined by PCR. The biofilm formation ability was evaluated and PFGE was performed to analyze for clonal relatedness among the isolates.
Results: Overall, 264 samples of all examined *Enterococcus* isolates and *E. faecalis* (50.0%) and *E. faecium* (22.3%) were the dominant species. Antimicrobial resistance rates were very low but the biofilm production was detected from 134 (50.6%) enterococcal isolates, especially in *E. faecalis* (95.5%). The PFGE results revealed that horse isolates were closely related to horse-associated environmental isolates in the same places.
Conclusion: Continuous monitoring was needed to prevent transmission to human by direct contact, since the spread of *Enterococcus* spp. between horses and environments was possible and half of enterococcal isolates had biofilm formation ability.

P1-ER05
Frequency and epidemiologic factors of extended spectrum beta lactamase positive uropathogenic *E. coli*
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Background: There is increasing incidence of ESBL producing *E. coli* causing community acquired urinary tract (UTI) infections. Bacteria that produce enzymes called extended-spectrum beta-lactamases (ESBLs) are resistant to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam. Infections with ESBL-producing organisms have been associated with poor outcomes. E. coli with ESBLs may cause urinary tract infections (UTIs) that can sometimes progress to more serious infections like urosepsis, which can be life threatening. Resistance makes these infections more difficult to treat. The aim of this study was to determine the frequency and epidemiologic factors of ESBLs positive *E. coli* in UTI.
Materials and Method: In this prospective study, from total of 501 community isolates of *E. coli* causing UTI, ESBL was detected by

CLSI criteria. Drug susceptibility was done by Kirby-Bauer method disc diffusion method for various antimicrobial agents. Various epidemiological factors associated with ESBL for each patient were recorded on individual forms. This included age, gender, antibiotics intake, history of urinary instrumentation, presence of diabetes mellitus, urolithiasis and recurrent UTI.

Results: Out of total of 501 strains of *E. coli*, which were screened for ESBL production, 62 (12.4 %) isolates were found to be positive. High-level resistance was seen for many antimicrobial agents like Amoxy-clavulanate (86.8%), Sulfamethoxazole-trimethoprim (55.1%), Nalidixic acid (54.9%), Cefotaxime (37.5%), Ciprofloxacin (37.2%), Norfloxacin (36.2%) and Cefixime (30.5%). Sensitivity to Nitrofurantoin was found to be 97.8% and only 2.2% of uropathogenic *E. coli* were resistant to Nitrofurantoin. Various epidemiological factors seen in ESBL producers include age >60 years (56.5%), female patients (85.5%), history of recurrent UTI (42 %), history of antimicrobial intake (58.1 %), diabetes mellitus (37.1%), history of urogenital instrumentation (29%) and urolithiasis (46.8%).

Conclusions: This study confirms that ESBL-producing *E. coli* strains are a notable cause of community onset infections especially in predisposed patients. The widespread and rapid dissemination of ESBL-producing *E. coli* seems to be an emerging issue worldwide.

Keywords: ESBL, Community acquired UTI, *E.coli*, Epidemiology

P1-ER06

In vitro activities of tedizolid and linezolid against Gram-positive cocci associated with acute bacterial skin and skin structure infections and pneumonia

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Background: Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) are of particular concern. The recent emergence of MRSA, coagulase-negative staphylococci, and VRE with reduced susceptibilities to linezolid has been widely reported. Tedizolid (formerly torezolid) is a novel, second-generation oxazolidinone with potent activity against a wide range of Gram-positive pathogens, including MRSA, VRE, *Streptococcus pneumoniae*, β-hemolytic streptococci, viridans group streptococci, and some linezolid-resistant strains.

Methods: A total of 425 isolates of Gram-positive bacteria were obtained consecutively from patients with ABSSSIs or pneumonia who were treated at National Taiwan University Hospital (NTUH) from January 2013 to October 2014.

Minimum inhibitory concentration (MIC) values of tedizolid and linezolid against the isolates were determined using the agar dilution method.

Results: Tedizolid exhibited 2-4 fold better *in vitro* activities than linezolid against methicillin-susceptible (n = 100), -resistant *Staphylococcus aureus* (n = 100), *Streptococcus pyogenes* (n = 50), *Streptococcus agalactiae* (n = 50), *Streptococcus anginosus* group (n = 75), *Enterococcus faecalis* (n = 50) and vancomycin-resistant *E. faecium* (VRE) (n = 50). Tedizolid MICs against *E. faecalis* (n = 3) and VRE (n = 2) intermediate to linezolid (MICs, 4 μg/ml) were 1 μg/ml and 0.5 μg/ml, respectively. Tedizolid MIC_{90s} against *S. anginosus*, *S. constellatus* and *S. intermedius* were 0.5, 0.5, and 1 μg/ml, respectively. Based on the proposed tedizolid MIC breakpoints by US FDA, all *S. aureus* isolates tested, including MSSA and MRSA, and 80% of *E. faecalis* isolates tested were susceptible to tedizolid. Overall, a large percentage (61.3%) of *S. anginosus* group isolates was not susceptible to tedizolid, particularly isolates of *S. angionsus* (84%) and *S. constellatus* (72%).

Conclusions: Tedizolid exhibited 2-4 fold better *in vitro* activities than linezolid against a variety of Gram-positive cocci associated with ABSSSIs and pneumonia. The unfavorable activities of tedizolid against isolates of *S. anginosus* and *S. constellatus* in Taiwan need further evaluation.

P1-GR01

Emergence of an imipenem sensitive meropenem resistant (ISMR) *Escherichia coli* and *Klebsiella pneumonia* in India

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Background: Carbapenem resistance among Enterobacteriaceae is an emerging problem worldwide. In general most widely used carbapenems, imipenem and meropenem have very similar sensitivity and resistant patterns against Enterobacteriaceae, predominately mediated by the production of carbapenem-hydrolyzing β-lactamases (carbapenemases). We recently noted the emergence of imipenem sensitive but meropenem resistant *Escherichia coli* (*E.coli*) and *Klebsiella pneumonia (K pneumonia) isolates* in India.

Methods: The study was conducted in Clinical Microbiology Lab at tertiary care referral hospital in South India, for a period of 1 year between January 2014 and December 2014. Imipenem and meropenem sensitivity for all *E coli and K pneumonia isolates* from clinical samples like pus, wound swab, sputum, endotracheal aspirate, blood and urine were analyzed. The antibiotic susceptibility testing was performed for these isolates by Kirby Bauer method, as per the latest CLSI guidelines.

Results: A total of 146 bacterial isolates of E coli (69) and K pneumonia (77) were analyzed during the study period. The sensitivity pattern of these isolates to imipenem and meropenem were shown in Table 1.

Table 1. *E coli* and *K pneumonia* sensitivity to imipenem and meropenem

	Imipenem sensitivity	Meropenem sensitivity
E coli (69)	66 (95.65%)	30 (43.47%)
K pneumonia (77)	69 (89.61%)	42 (54.54%)

Conclusion: Most of the E coli and K pneumonia were sensitive to imipenem but resistant to meropenem. Laboratories that use meropenem as marker of carbapenem resistance may overestimate the carbapenem resistance. There is an urgent need to identify the resistance mechanism responsible for this sensitivity pattern to decide future treatment options for E coli and K pneumonia infections in India.

P1-GR02

Increasing quinolone resistance in *E. coli* and *K. pneumoniae* isolated from urine culture at emergency room in children young than 24 months old: a retrospective single center study for 15 consecutive years

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Objective: To investigate any quinolone resistance in gram negative bacteria in children who have little exposure to quinolones in addition to usual antimicrobials typically used in treating urinary tract infection (UTI) in children.

Method: A retrospective chart review was conducted in pediatric patients younger than 24 months 0 days who visited emergency room at our institution. We selected urine culture data that grew *E. coli* and *K. pneumoniae*. Baseline clinical information and results of antimicrobial susceptibility test were analyzed by dividing 15-year study period into three periods (A: 2000-2004, B: 2005-2009, and C: 2010-2014).
Results: During study period, 891 children were identified. *E. coli* was isolated in 84.2% (750/891) of patients and *K. pneumoniae* was isolated in 15.8% (141/891). Median age of patients was 0.4 years (range, 6 days to 1.9 years), and the male was 68.6%. Antimicrobial resistance rate of ampicillin/sulbactam was 67.2% (248/369), cefotaxime 8.8% (60/681), piperacillin/tazobactam 12.8% (111/862), trimethoprim/sulfamethoxazole 35.8% (316/882), and amikacin 2.1% (19/891). Resistance rate of quinolones (ciprofloxacin or levofloxacin) was 7.5% (67/890). Quinolone resistance rates significantly increased in later years from period A to period C (A, 7.6% (22/286); B, 11.5% (30/262); C, 14.1% (48/342), $P = 0.021$).
Conclusion: This study revealed that common uropathogens, *E. coli* and *K.pneumoniae* isolated from urine culture in young children less than 24 months had increasing resistance rates against quinolones.

P1-GR03
Extended-spectrum-cephalosporin-resistant urinary *Enterobacteriaceae* in children younger than 2 years of age: a retrospective single center analysis

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Background: Urinary tract infections are among the most common bacterial infections in children. Antimicrobial resistance in *Enterobacteriaceae* is a growing concern. The objective of this study is to investigate the antimicrobial susceptibility of *E. coli* and *Klebsiella* species isolated from urine specimens representing community-acquired infections.
Methods: A retrospective review was performed in patients (1 to 23 months of age) with *Enterobacteriaceae* isolated from urine cultures at Hallym University Dongtan Sacred Heart Hospital from August 2013 to July 2014. Extended-spectrum-cephalosporin-resistant (ESC-R) isolate was defined as nonsusceptibility to cefotaxime or ceftazidime. The AmpC phenotype was defined as nonsusceptibility to cefotaxime or ceftazidime and nonsusceptibility to cefoxitin.
Results: One hundred forty-eight urinary isolates were identified: 108 (73.0%) *E. coli*, 25 (16.9%) *Klebsiella* species, 8 (5.4%) *Enterobacter* species and 5 (3.4%) *Citrobacter* species. Among *E. coli* and *Klebsiella* species (133/148, 89.9%), ESC-R isolates were 32 (32/133, 24.1%): 20 (20/133, 15.0%) ESBL-producing isolates; 8 (8/133, 6.0%) AmpC phenotype; 4 (4/133, 3.0%) ESBL producing isolates with AmpC phenotype. All ESC-R *E. coli* and *Klebsiella* species were susceptible to amikacin. The susceptibility to ciprofloxacin and trimethoprim-sulfamethoxazole were 62.5% (20/32) and 43.8% (14/32), respectively. Among ESBL-producing isolates, the susceptibility to beta-lactamase inhibitors was 45.0% (9/20) for amoxicillin/clavulanate and 90% (18/20) for piperacillin/tazobactam.
Conclusion: In this study, the frequency of ESC-R strains in *E. coli* and *Klebsiella* species isolated from urine specimens was 24.1% and the level of amikacin susceptibility was high.

P1-GR04
Identification and characterization of IMP-1 metallo-β-lactamase producing *Acinetobacter* species from non-tertiary hospitals

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Background: *Acinetobacter* spp. is non-fermenting gram-negative bacilli and known as one of the major health care associated infectious pathogens. Increasing carbapenem resistance in Gram negative bacteria including *Acinetobacter* spp. has been becoming a critical worldwide problem in the clinical field. Carbapenem-resistance in Acinetobacter spp. is caused mainly by carbapenem-hydrolysing class D β-lactamase (CHDL) and rarely metallo β-lactamase (MBL). We identified IMP-1 β-lactamase producing *Acinetobacter* spp. isolates collected from non-tertiary hospitals and report it here.
Methods: Non-baumannii *Acinetobacter* spp. was isolated from 305 putative *A.baumannii* isolates collected from non-tertiary hospitals during 2012 and 2013. After differentiation of isolates from *A. baumannii* by identifying their possession of OXA-51-like gene using PCR, identification of each bacterial species was confirmed by comparing of 16S rRNA and *rpoB* sequencing. Carbapenem resistance was confirmed by estimating their MIC. Carbapenemase activities were tested by modified hodge test (MHT) and double-disk synergy (DDS) test. The existence of CHDL and MBL(bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SIM}, and bla_{SPM}) genes were verified by PCR and sequencing.
Results: Single IMP-1 carbapenemase-producing from non-baumannii *Acinetobacter* spp. was identified in this experiment. The strain was identified as *A. bereziniae* (or *Acinetobacter* gen. sp. 10). This strain was susceptible to colistin and tigecyclin, and non-susceptible to antibiotics including imipenem, meropenem, ampicillin-sulbactam, piperacillin-tarzobactam, ciprofloxacin, cefepime, and ceftazidime.
Conclusion: Although it is known that there are little MBL than the OXA family of β-lactamase in carbapenem-resistant *Acinetobacter* spp., the appearance of MBL-producing *Acinetobacter* spp. in general hospitals were recently reported. And MBL-producing *Acinetobacter* spp. is appeared even if the isolates from non-tertiary hospitals. Extensive epidemiological concerns should be going on dissemination of transferable β-lactamase genes in *Acinetobacter* spp.

P1-GR05
Study the invitro effectivity of colistin in combination with imipenem and meropenem on the multidrug-resistant *Acinetobacter baumannii*

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Background: *Acinetobacter baumannii* is one of the leading causes of hospital-acquired infections. Today, management of multidrug-resistant *Acinetobacter* spp. infections are a great challenge for physicians and clinical microbiologists. In treatment of infections caused by *Acinetobacter baumannii*, colistin is often used with meropenem or imipenem, but in vitro experiments, figures on synergistic effects when used in combination antibiotic colistin with meropenem or imipenem have not shown out.
Objectives: Study on effectively of colistin in combination with other antibiotics on isolates of multidrug-resistant *Acinetobacter baumannii*.
Method: 100 Bauamnnii Acinetobacter strains isolated from clinical specimens of patients with different infections at Nguyen

Tri Phuong Hospital. Performing antimicrobial susceptibility testing to determine antimicrobial minimum inhibitory concentrations (MIC) using microdilution method on meropenem, imipenem, colistin and some other antibiotics. Then assessing the effectiveness of antibiotic combination between colistin with meropenem or imipenem by the chessboard method to find out the synergistic effects of colistin at different concentrations below the MIC with meropenem or imipenem.

Results: *A. baumannii* is highly resistant to many antibiotics, typically with imipenem (75%), meropenem (79%), and relatively low with colistin (7%). When colistin at concentrations of 0.125 μg/mL, 0.25 μg/mL and 0.5 μg/mL and below its MIC were combined with meropenem the synergistic effect increases respectively of 62%, 70% and 88%; and with imipenem, the synergistic effect also increases respectively of 18%, 38% and 95%. In addition, colistin at a concentration of 0.5 μg/mL, and lower that its MIC, can make 66% of meropenem resistant *A. baumannii* become susceptible to meropenem and 65% of imipenem resistant *A. baumannii* becoming susceptible to imipenem.

Conclusion: *A. baumannii* has the high ratio of resistant to many antibiotics including imipenem and meropenem, but still susceptible to colistin. Colistin is likely to increase the synergistic effect of meropenem or imipenem at the usual dose, and reduce the rate of resistance to meropenem and impenem on *A. baumannii* when combined with it.

P1-GR06

In vitro activity of polymyxin B, tigecycline, meropenem and aztreonam against OXA-181 producing _Klebsiella pneumoniae_

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Background: The prevalence of Carbapenem-resistant Enterobacteriaceae (CRE) has been increasing worldwide. Polymyxin and Tigecycline are often used as the last resort antibiotics against CRE. Meropenem and Aztreonam are reported to have variable susceptibility towards OXA-181 which is a variant of Class D carbapenemase. Due to the lack of new antimicrobial agents to treat this bacterial infections, combination antimicrobial agents may be the only therapeutic option until new drugs are available. Therefore, we aim to evaluate the in-vitro activity of Polymyxin B, Tigecycline, Meropenem and Aztreonam alone and in combination against OXA-181 producing Klebsiella pneumoniae (KP).

Methods: 4 well characterized OXA-181 producing KP were studied. Minimum inhibitory concentrations (MIC) of the isolates were determined by E-test. Time-kill (TK) assay were conducted with approximately 5 log CFU/mL at baseline using maximum clinical achievable concentrations (mg/L) of Polymyxin B (2), Tigecycline (2), Meropenem (64) and Aztreonam (48) against the 4 isolates. Bactericidal activity is defined as ≥3 $\log_{10}$ reduction in the total CFU/mL from the original inoculum. Synergy is defined as ≥2 $\log_{10}$ decrease in the number of CFU/mL between the combination and the most active compound and a ≥2 $\log_{10}$ CFU/mL decrease from the initial inoculum at 24 hour.

Results: All 4 isolates were resistant to Meropenem and Aztreonam except for Polymyxin B (MIC 0.38-0.5 mg/L) and Tigecycline (0.5-4 mg/L). TK assay showed only 2 isolates exposed to Polymyxin B were bactericidal with the exception of Tigecycline, Aztreonam and Meropenem. Polymyxin B plus Tigecycline exhibited bactericidal activity with the range from 3.02 to 5.56 $\log_{10}$ CFU/mL (mean, 4.77 $\log_{10}$ CFU/mL) against all 4 strains. Polymyxin B plus Meropenem combination exhibited bactericidal activity against 3 isolates which yielded a log kill of 5.86, 5.96 and 5.85. Only 1 isolate had initial reduction to 0 CFU/mL until 8 hour before regrowth happened at 24 hour. Initial decline of inocula were observed in 2 isolates after being exposed to Tigecycline plus Meropenem but had regrowth by 24 hour. Neither the Tigecycline plus Meropenem nor the Tigecycline plus Aztreonam combinations exhibited bactericidal activity.

Conclusion: Bactericidal activity and synergistic effect between Polymyxin B and Tigecycline were observed in most isolates tested. This combination may be clinically useful in the treatment of infections caused by OXA-181 producing KP. Polymyxin B monotherapy, Tigecycline plus Meropenem and Aztreonam containing combinations may not be appropriate for infection caused by OXA-181 producing KP.

P1-GR07

Spread of multidrug-resistant _Pseudomonas aeruginosa_ with ST235 clone in Korean hospitals, 2003-2009

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Background: Multidrug-resistant (MDR) *Pseudomonas aeruginosa* has increased in Korean hospitals over the years and carbapenem-resistant *P.aeruginosa* has also been spreading nationally. In this study, we investigated the dissemination of metallo-β-lactamase (MBL)-encoding genes and the genetic diversity among MDR *P.aeruginosa* isolates in different Korean hospitals.

Methods: We used 655 *P.aeruginosa* isolates from tertiary (n = 290, 44.3%), non-tertiary (n = 248, 37.9%) and geriatric (n = 117, 17.9%) hospitals between 2003 and 2009. The standardized disk diffusion test and E-test were used to determine resistance to antibiotics. MBL-encoding genes were detected by multiplex PCR. Clones were defined by pulsed-field gel electrophoresis (PFGE) of *Xba*I DNA digests. Selected strains by PFGE types were characterized by multilocus sequence typing (MLST).

Results: Of all isolates, 32.4% (n = 212) were MDR strains showing resistance to fluoroquinolones, aminoglycosides, and β-lactams in the period 2003-2009. Resistant to carbapenems was increased over this period from 6.8% to 50.5%. MBL-producing *P.aeruginosa* was detected in 80 isolates ($bla_{\text{IMP-1}}$ (n = 2), $bla_{\text{IMP-6}}$ (n = 45), and $bla_{\text{VIM-2}}$ (n = 33)). All isolates with $bla_{\text{IMP-}}$ genes were clustered as the same PFGE type with a single major clone, ST235. Whereas $bla_{\text{VIM-2}}$ producers had distinct PFGE patterns broadly.

Conclusion: MBL-producing MDR *P.aeruginosa* isolates have been increased in Korean hospitals since 2003. The IMP- and VIM-producers belonging to the most common international clone (ST235) were spread throughout the country.

P1-GR08

Contamination and antibiotic resistance genes of _Escherichia coli_ and _Staphylococcus aureus_ in vegetables and farm related samples in South Korea

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South Koreans consume a wide variety of fresh vegetables, even though fresh vegetables are one of major cause of foodborne diseases. Amongst OECD countries, Korea has one of the highest antibiotic resistance rates. *Escherichia coli* and *Staphylococcus aureus* are used to assess the hygiene level of food and are well-known as disseminators of antimicrobial resistance genes. The purpose of this report is to show the contamination level and antibiotic resistance of *E.coli* and *S. aureus* isolated from vegetables and farm environmental samples.

Total 1070 samples were collected from 116 farms in eight provinces in Korea. Disk Diffusion and Minimum Inhibitory Concentration experiments were conducted to show antibiotic resistance against 21 antibiotics, 9 classes. And also to check phylogenetic groups (chuA, yjaA, TspE4.C2), virulence genes (ipaH, stx1, stx2, lt, eaeA, bfpA, aggR), and other antibiotic resistance genes via Polymerase chain reaction.

Result*: E.coli* isolates showed high resistance to cephalosporins, especially first generation and about half of the phylogenetic group were B1. *S.aureus* isolates showed high resistance to penicillins and rifampicin.

The results of this study shows that *E.coli* could be a donor of antibiotic resistance genes to pathogenic foodborne bacteria. It is necessary to mention that regulations should not only be in place to monitor animal feeds and pesticides, however it is necessary to introduce more steps to reduce the risk and spreading of antibiotic resistance.

P1-GR09

Characterization of veterinary hospital-associated antimicrobial-resistant *Escherichia coli* isolates in Korea

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Background: *Escherichia coli* (*E. coli*) might be a significant reservoir of antimicrobial-resistance can spread antimicrobial-resistance genes to another bacteria and previous studies have reported an increased level of resistance in *E. coli* isolates to diverse antimicrobials for the treatment of companion animals. For this reason, it has been hypothesized that antimicrobial-resistant (AR) *E. coli* are widely disseminated in veterinary hospitals in Korea.

Methods: Isolated putative colonies from 294 swab samples were from dog patients and their owners confirmed as *E. coli* by PCR method and antimicrobial sensitivity of *E. coli* isolates was determined by a disk diffusion test with the 14 antimicrobial disks according to the CLSI. PCR was performed to all isolates resistant to ampicillin, tetracycline, chloramphenicol and sulfamethoxazole/trimethoprim for screening the presence of the individual resistance genes. The genetic relatedness among the AR *E. coli* isolates was determined by PFGE.

Results: A total of 39 *E. coli* isolates (39/294; 13.3%) were obtained from the swab samples and 19 isolates (19/39; 48.7%) were resistant to at least one antimicrobial agent. Most of AR *E. coli* isolates carried the corresponding resistance genes with minor exceptions. PFGE analysis with the 19 AR *E. coli* isolates showed that 2 clone sets identical in their molecular patterns were isolated from different dog patients in same hospital.

Conclusion: The PFGE data indicated the possibility for cross-transmission of AR *E. coli* clones between dog patients. Therefore, we should examine the prevalence and antibiogram of veterinary hospital-associated *E. coli* constantly.

P1-GR10

Resistant *Escherichia coli* in irritable bowel syndrome – cause or effect?

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Background: Irritable bowel syndrome is increasingly recognized all over the world. There are evidence for an association between Intestinal commensal microbiota and irritable bowel syndrome, though the exact relationship is still not well understood. Antibiotic sensitivity of bacteria is determined genetically and influence d by the environment. As the physico-chemical environment of the gut is altered in irritable bowel syndrome, antibiotic sensitivity patterns may also be altered. Recognition of such patterns will provide some insight for the pathophysiology of the disease.

Methods: The objective of this study is to describe antibiotic sensitivity patterns of *Escherichia coli* in commensal flora of patients with irritable bowel syndrome. Newly diagnosed patients with irritable bowel syndrome and age and gender matched control group were included in the study. Antibiotic sensitivity of *Escherichia coli* isolated from stool cultures was analyzed to detect extended spectrum beta lactamase production.

Results:

	IBS +	IBS -	Total
ESBL	14	03	17
Non ESBL	08	19	27
Total	22	22	44

The Chi-square statistic is 11.5991. The P value is 0.00066

Conclusion: There is a statistically significant (P value 0.00066) association between ESBL Ecoli and Irritable bowel syndrome in this patient cohort. This may be related cause or effect of the disease process. Multicenter study with a bigger patient cohort will provide more reliable information.

P1-GR11

Emergence of gentamycin resistant *Salmonella enterica* serotypes virchow in diarrhoeal hospitalized patients in Delhi, India during 2013-2014

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Aim and objective: To study the non typhoidal *Salmonella* among diarrhoea cases and to co-relate the drug resistance of *Salmonella enterica* serotype Virchow is an etiological agent of non typhoidal *Salmonellosis* an emerging trend observed in diarrhoeal disease surveillance.

Materials and methods: During 2013-2014 we have isolated 15 cases of *Salmonella enterica* serotype Virchow from stool samples of patients admitted in Maharishi Valmiki Infectious Diseases Hospital (MVIDH), Kingsway Camp, Delhi, India. Rectal swab samples were processed as per WHO guidelines (CDC/WHO/USAID, 2003).

Antibiotic sensitivity test and MIC

Antimicrobial sensitivity and MIC of all 15 *Salmonella enterica* serotype Virchow isolates were carried out by Kirby-Bauer ((Bauer et al., 1966; CLSI, 2013) disk diffusion method using commercially available antibiotic disks and minimum inhibitory concentration (MIC) was carried out by agar dilution method as per CLSI guidelines 2013(CLSI, 2013).

Results: Conformation of isolates.

All 15 *Salmonella enterica* serotype Virchow was serologically confirmed *Salmonella enterica subsp. enterica* serovar Virchow (6,7,r:1,2) according to White-Kauffmann-Le Minor scheme.

Antibiotic sensitivity assay

All *Salmonella enterica* serotype Virchow were 100% resistant to amikacin, gentamicin, nalidixic acid followed by cefotaxime, ciprofloxacin and 100% sensitive to ampicillin, norfloxacin, ceftriaxone, chloramphenicol, and imipenem. The MIC of gentamicin resistant isolates having MIC of ≥512 μg/ml.

Conclusion: In 2013 we have only got two *Salmonella enterica* serotype Virchow but in 2014 suddenly it emerged in Delhi NCR and all are gentamicin and amikacin resistant. The same clone emerged in both humans and poultry, indicating that poultry was

probably the main source for *Salmonella* serovar Virchow in the food chain. Heightened awareness and coordinated approach by national and international health, food, and agriculture authorities is necessary to implement measures to monitor and limit spread of this strain.

Acknowledgement: We are grateful to Principal, A.N. College, Patna and HOD of Zoology Department .We are thankful to Lab staff of MVID, Hospital. We also thankful to Mr. Naveen Saini for secretarial assistance. Dhirendra Kumar is thankful to UGC, Government of India for junior research fellowships under Rajiv Gandhi National Fellowship.

Keywords: *Salmonella* Virchow, Gentamycin resistant, MIC, Delhi-NCR, MVIDH

P1-GR12

Prevalence of ESBL and MBL encoding genes in *Acinetobacter baumannii* strains isolated from patients of intensive care units (ICU)

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The aim of this study was to investigate the prevalence of ESBL and MBL encoding genes among *A. baumannii* isolates. In this cross sectional study, 100 *A. baumannii* strains were isolated from ICU wards of 3 educational hospitals of Hamadan City, Iran in 2011. Phenotypic identification of the production of ESBLs and MBLs has been carried out by using E-test and DDST methods, respectively. PCR technique was used for amplification of the ESBL and MBL encoding genes, namely: CTX-M, SHV, TEM, OXA-51, VIM-Family, IMP-Family, SPM-1, SIM-1, and GIM-1. Eighty seven (87%), 95 (95%), 98 (98%) and 95 (95%) out of 100 *A. baumannii* isolates were resistant to imipenem, meropenem, ceftazidime and cefotaxime, respectively. Also, 99% and 7% of the isolates were MBLs and ESBLs produced phenotypically. Thirty (30%), 20 (20%) and 58 (58%) out of 100 *A. baumannii* isolates have been confirmed to harbor the bla_{VIM}-family, TEM and SHV genes, respectively. Our results show no significant relationship between the detected gens with production of MBLs and ESBLs in spite of high prevalence of MBL encoding and drug resistant *A. baumannii*. Probably some other genes rather than what we studied are involved in phenotypic production of MBLs and ESBLs and subsequent drug resistance in Hamadan area, Iran.

Keywords: *Acinetobacter baumannii*; Drug resistance; MBL; ESBL

P1-GR13

Multidrug resistant and carbapenemase producing *Enterobacteriaceae* among patients with urinary tract infection at referral hospital, Northwest Ethiopia

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Background: Increased burden of multidrug resistant Enterobacteriaceae (MDRE) causing urinary tract infection (UTI) compounded by harboring carbapenemase producing strains becomes a serious threat to public health. Carbapenemase producing Enterobacteriaceae (CPE) expresses enzymes that can break down carbapenems. Prevalence of MDRE in different part of the world is increasing, but data about the incidence of CPE in is not yet documented in Ethiopia.

Objective: The aim of the study was to assess the prevalence and risk factors of MDR and CPE among patients with UTIs.

Methods: A cross sectional study was conducted among 442 symptomatic UTI suspected patients at the University of Gondar Hospital from February to May 2014. Systematic random sampling technique was used to select the participants. Data on socio-demographic characteristics, clinical information and possible risk factors were collected using structured questionnaire. Mid-stream urine samples were collected and processed to characterize bacterial isolates. Disk diffusion method was used to determine the antibiotic susceptibility patterns of isolates. In this particular study, CPE isolates were detected using CHROMagar KPC medium. Data were entered and analyzed using SPSS version 20. P-value <0.05 were considered as statistical significant.

Results: A total of 442 patients with mean age of 37.1 years were included in this study and the majorities were females (63.8%). From 183 (41.4 %) of patients, 183 Enterobacteriaceae isolates were identified; of which, 160 (87.4%) were MDRE; the principal isolates were *E.coli* and *K. pneumoniae*. Moreover, 5 (2.73%) of isolates were found to be carbapenemase producers, namely *E.coli* (2), *K. pneumoniae* (2), and *E. aerogenes* (1). Significant drug resistances were observed among CPE compared to other MDRE, low resistance rates were noted to ciprofloxacin (20%). Being female (OR 4.46; P = 0.018), age (OR 1.08; P = 0.001), hospitalization (OR 5.23; P = 0.006), and prior antibiotic use (OR 3.98; P = 0.04) were associated risk factors with MDRE.

Conclusion and recommendations: Increased prevalence of MDRE and incidence of CPE were indicated in this study. Attributing risk factors for MDRE were found to be sex (female), age, hospitalization, and history of antibiotic therapy. Therefore, efforts should be directed to reduce patient hospital stay and to maximize rational use of drugs. Additional and vigorous investigation especially on CPE should be encouraged.

Key words: Carbapenemase, Enterobacteriaceae, Multidrug resistant, Urinary tract infection

P1-GR14

Characteristics of carbapenem-resistant *Acinetobacter spp.* isolates in South Korea, 2014

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Background: Carbapenem has become drug of choice for *Acinetobacter spp* .infection due to its resistance to multiple classes of antibiotics. However, Carbapenem resistant rates in *Acinetobacter spp.* are increasing around the world. The aim of this study was to examine the antibiotic resistance rates and molecular mechanism of carbapenem resistance in clinical *Acinetobacter spp.* isolates in South Korea, 2014.

Methods: From March 2014 to July 2014, a total of 336 non-duplicate isolates of *Acinetobacter spp*. were collected from 24 different hospitals in 6 major cities/provinces of Korea. Identification of strains was performed using MALDI-TOF MS system. Antimicrobial susceptibility was initially determined by BD Phoenix automated system. MIC of carbapenem was determined using Etest strip and simplex PCR assay was used for detection of carbapenem resistant genes.

Results: A total of 304 *A.baumannii* and 32 non-*baumannii Acinetobacter* (NBA, 17 *A.nosocomialis,* 11 *A.pitti*, 2 *A.calcoaceticus* and each of 1*A.junii, A.soli*) were identified. While 11% (4/33) of NBA isolates exhibited resistance to imipenem and/or meropenem, 90% (273/304) of *A.baumannii* were resistant to these drugs. In *Acinetobacter baumannnii*, bla_{oxa-23}-like gene was detected in 264/273 (97%), IS*Aba1*-associated bla_{oxa-51}-like gene was detected in 7/273(3%). Two isolates coexpressed the bla_{oxa-23}-like gene and IS*Aba1*-associated bla_{oxa-51}-like gene. In three carbapenem-non-susceptible NBA isolates, bla_{oxa-23}-like gene, bla_{oxa-58}-like gene and bla_{IMP-1} were detected individually.

Conclusion: We confirmed high carbapenem resistant rates among *Acinetobacter baumannii*. Also, bla_{oxa-23}-like gene and IS*Aba1*-associated bla_{oxa-51}-like gene were main molecular mechanism of carbapenem resistance in South Korea, 2014.

P1-GR15

Direct detection and spreading of mosaic *penA*, which is associated with cephalosporin resistance in Korea

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Background: The emergence and spread of cephalosporin-resistant *N. gonorrhoeae* has become a serious problem worldwide. However, the recent introduction of molecular diagnosis for gonococcal infection may be a handicap for performing the antimicrobial susceptibility test for appropriate treatment of resistant isolates. We aimed to determine, using molecular assay, the prevalence of *penA* mosaicism, which was responsible for cephalosporin resistance in clinical specimens, and to investigate the epidemiologic relationship of specimens positive for this molecular method.
Methods: A total of 1561 DNA specimens that were confirmed as *N. gonorrhoeae* infections by PCR were collected from two commercial laboratories across the nation from 2012 to 2014. DNA specimens were tested for the modified *penA* genes responsible for the resistance of cefixime or ceftriaxone in two steps: screening for mosaic *penA* and detecting *penA* types, which are response for high-level ceftriaxone resistance, by real-time PCR, high resolution melting analysis, and sequencing. *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) and multilocus sequence typing (MLST) were carried out for all available DNA specimens to determine the molecular epidemiologic relatedness.
Results: The positive rate of DNA specimens in mosaicism screening was 1.1% (5 of 433) in 2012, 6.7% (35 of 524) in 2013, and 9.9% (60 of 604) in 2014. The increase of mosaic *penA* started in Seoul/Gyeonggi province and Busan/South Gyeongsang province and then disseminated to other regions. No screening-positive DNA specimens bore the *penA* gene of H041 and F89 strains, based on real-time PCR and Mosaic501-hybPRC analysis. In *penA* gene sequencing, type X was most common (84 of 100), and type XXXIV with P551S substitution (5 of 100) and type XXXIV (3 of 100) were also detected with three novel *penA* types. The most prevalent STs by NG-MAST were changed from ST2958 in 2012 and 2013 to ST10668 in 2014. On phylogenetic tree analysis, most *por* genes noted from isolates bearing type X mosaicism showed genetic relatedness, and most specimens belonged to ST1901, according to MLST.
Conclusion: It may be assumed that the dissemination of *N. gonorrhoeae* isolates with *penA* alterations responsible for cefixime resistance is in progress in some areas in Korea due to increased selective pressure, and some alterations have the capability of evolving to achieve high-level ceftriaxone resistance.

P1-GR16

Clinical impact of the eleven strategies introduced to combat antimicrobial resistance rate

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Background: Considering that antibiotics were introduced into medicine not even hundred years ago it is an utter challenge to resist the impending threats of Carbapenem Resistant Gram Negative Infections (CRGNB). The project was undertaken to mitigate the Patient Safety related risk from these infections in the community/outside hospital acquired infection coming to Apollo Gleneagles Hospitals for further management and care. This sudden rise of CRGNB patients (30 in Feb 2012 compared to 8 in Jan 2012) with an attributable mortality of 16.6% (5/30 patients) posed a serious patient safety challenge to control in a cost-effective manner with reinforcing existing practices and innovating others, to reduce the mortality and morbidity associated with the killer organisms.
Methods: Eleven strategies were implemented to achieve our goal of Zero in-house infection on CRGNB. The strategies included improvement in hand hygiene, use of appropriate PPE, handling of soiled patient care equipment, stringent environmental controls, handling of textile and laundry, daily 2% Chlorhexidine bath, prioritization of patient placement, management of patient transport (Code Green), surveillance samples, introduction of daily tracking dashboard and justification of use of restricted antibiotics.
Results: Before interventions, environmental samples showed 17% CRGNB contamination (Mar 2012) which was brought down to 0% (Apr 2012). The attributing mortality came down to 6.67% from February through May. There was zero in-house infection on CRGNB by Mid May 2012.
Conclusion: Antimicrobial Resistance continues to be a public health concern with limited treatment options and huge economic burden. Reducing length of stay in hospitals, operational monitoring of environmental and patient specific controls and prudent use of antibiotics can collectively address the root cause of this major global concern.

P1-GR17

Emergence of fluoroquinolone-resistant *Stenotrophomonas maltophilia* in blood isolates causing bacteremia: molecular epidemiology and microbiologic characteristics

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Background: *Stenotrophomonas maltophilia* is an important nosocomial pathogen associated with bacteremia, respiratory tract infections, and urinary tract infections. *S. maltophilia* is intrinsically resistant to broad-spectrum antibiotics, including most β-lactams and aminoglycosides, and clinical isolates exhibits high-level multidrug resistance. Sulfamethoxazole-trimethoprim (SMX-TMP) and fluoroquinolones (FQ) are common antibiotics used to treat *S. maltophilia* infections. However, *S. maltophilia* resistance to fluoroquinolones, especially to levofloxacin, has been increasing. The purpose of this study was to determine antimicrobial susceptibilities of recent clinical *S. maltophilia* isolates causing bacteremia and to characterize the quinolone resistance-determining regions (QRDRs) of *gyrA*, *gyrB*, *parC*, and *parE* in levofloxacin-resistant isolates.

Methods: *S. maltophilia* isolates causing bacteremia were collected from January 2006 to March 2014 at 4 tertiary-care hospitals in Korea. In the antimicrobial susceptibility tests, minimum inhibitory concentration (MIC) was determined by using the broth microdilution method and an E-test in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. The genetic relationship of the levofloxacin-resistant *S. maltophilia* isolates was assessed using multilocus sequence typing (MLST). To investigate the presence of mutations in the QRDRs, the chromosomal genes were PCR amplified and sequenced.
Results: Among a total of 127 clinical isolates included in this study, 41 (32.2%), 63 (49.6%), and 65 (51.1%) were non-susceptible to levofloxacin, ceftazidime, and ticarcillin/clavulanic acid, respectively. All isolates were susceptible to minocycline. The MIC_{90} were 64 mg/L for colistin, 32 mg/L for ciprofloxacin, 16 mg/L for levofloxacin and moxifloxacin, and 8 mg/L for gemifloxacin. The MIC_{90} of tigecycline was 32 mg/L, which was four-fold higher than that of minocycline. Only three isolates (2.3%) were resistant to SMX-TMP. The most prevalent sequence type (ST) was ST77, which included 8 isolates (19.5%), followed by ST28 (3 isolates, 7.3%). In this study, 17 STs (ST97 to ST113) were newly identified. Amino acid substitutions were found in the *gyrB* and *parC* with 2 and 10 isolates, respectively, compared with the corresponding sequences of *S. maltophilia* ATCC 13637. None of the isolates harbored amino acid substitutions in the *gyrA*. Twenty-three isolates showed amino acid substitution in the *parE* gene. Of these, both Met437→Leu and Ile465→Val substitutions were observed in 17 isolates. But these changes were not linked with high levofloxacin MICs.
Conclusion: FQ-resistant *S. maltophilia* isolates have emerged and disseminated in Korean hospitals, even in blood isolates causing bacteremia. The high frequency of amino acid replacements were present in the *parE*. However, these specific replacements were not correlated with high levofloxacin MICs.

P1-GR18

A plasmid bearing blaCTX-M-15 gene and phage P1-like sequences from ST11 *Klebsiella pneumoniae* isolate

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Objectives: This work attempted to characterize a plasmid carrying $bla_{CTX-M-15}$ from *Klebsiella pneumoniae* ST11, a clone rapidly disseminating worldwide.
Methods: A plasmid pKP12226 was extracted and analyzed from a *K. pneumoniae* ST11 isolate collected in Korea. The *K. pneumoniae* isolate has been characterized to produce CTX-M-15-type extended spectrum β-lactamase to be resistant to most antimicrobial agents, and to bear a plasmid of the IncFII group. Plasmid DNA was sequenced using a 454-Genome Sequencer FLX system and aligned against existing GenBank data.
Results: The pKP12226 plasmid encodes antibiotic resistance genes to β-lactams ($bla_{CTX-M-15}$ and bla_{TEM}), tetracyclines (*tet(A)* and *tetR*), aminoglycosides (*aadA2*), trimethoprim (*dfrA17*), sulfonamide (*sul1*), macrolides (*mph*(A)-*mxr*-*mph*(R)) and quaternary ammonium compounds (*qacEΔ1*). Four addiction systems (*mok*/*hok*, *ccdA*/*ccdB*, *vagCD* and *pemKI*) were identified, ensuring stabilization of the plasmid in host bacteria. The plasmid consists of a backbone that is highly similar to the pIP1206 plasmid from *Escherichia coli* isolated in Belgium and a resistance region that is different from previously characterized plasmids bearing $bla_{CTX-M-15}$ from Korea. In addition, we identified lysogenized phage P1-like sequences in the plasmid.
Conclusion: The plasmid pKP12226 from the *K. pneumoniae* ST11 isolate may have resulted from recombination between an *E. coli* plasmid backbone, a $bla_{CTX-M-15}$-bearing resistance region, and lysogenized phage P1-like sequences. It may have originated differently from plasmids of other prevailing CTX-M-15-producing *K. pneumoniae* clones in Korea.

P1-GR19

Antimicrobial resistances and genotypes of carbapenem-resistant gram-negative bacterial isolates from Korean carriers

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Objective: The global emergence and dissemination of carbapenem-resistant Gram-negative bacteria (CR-GNB) has posed great concern to public health.
Methods: Carrier samples were collected from 300 individuals with health medical examination in October 2013 in Korea. The carrier samples are plated on MacConkey agar selective medium with 2 mg/L imipenem for selection of gram-negative organism. Species identification was performed by 16S rRNA gene sequence analysis. In vitro antimicrobial susceptibility testing and efflux pump activity were performed. Genetic relationships among isolates were assessed by multilocus sequence typing (MLST) or pulsed-field gel electrophoresis (PFGE). The screening for carbapenemase activity in these isolate was detected using the EDTA-imipenem disc synergy test and carba-NP test. MBL genes were detected by PCR assay.
Results: CR-GNB isolates were identified in 79 (26.3 %) out of the 300 individuals investigated. In 79 samples, a total of 83 CR-GNB isolates were identified. Two or three different species of CR-GNB were identified in three samples. Among 14 species identified, *Proteus mirabilis* was the most frequent (32 isolates), followed by *Pseudomonas aeruginosa* (13 isolates), *Morganella morganii* (11 isolates), *Stenotrophomonas maltophilia* (8 isolates). Of these, eight *S. maltophilia* isolates (9.6%) produced carbapenem-hydrolyzing L-1 β-lactamase. PFGE and MLST showed a higher genetic diversity among *P. mirabilis, P. aeruginosa,* and *S. maltophilia* isoaltes
Conclusion: In this study, we identified high carriage of CR-GNB isolates in healthy Korean. In addition, diverse genotypes of CR-GNB isolates was identified.

P1-GR20

A glimpse into bacterial behaviour in the era of NDM1

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Background: Over the last few years, the world has witnessed a dramatic change in the antimicrobial resistance trend. India is considered to be on the forefront of this change, with NDM-1 producing bacteria dominating the picture.
Methods: Retrospective analysis of the susceptibility of Gram negative isolates from a tertiary care neurosurgical and oncology centre and bone marrow transplantation in South India. Carbapenam resistance (CR) of *Pseudomonas, Klebsiella, E.coli* and *Acinetobacter* isolates from all inpatient samples from January 2010- December 2014 were analysed, using Vitek autonalyser and MiniAPI. Carbapenam resistance of 5861 isolates (*E coli*- 1616, *Klebsiella*-2027, *Acinetobacter* -545, *Pseudomonas*-1693) were separately looked into to analyse the trend of resistance.
Results: There was a steep increase in CR *E.Coli* and *Klebsiella* from 2010 to 2012 (1-12%, 2%-33% respectively). This corresponds to

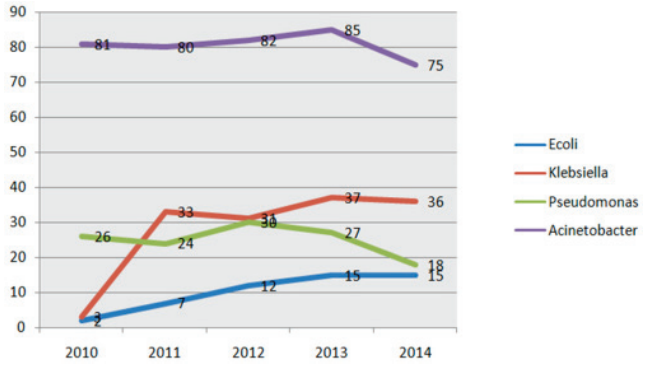

Fig. 1. Carbapenem resistance trend over 5 years.

the NDM-1 crisis in South Asia, which peaked during the period. Since 2012 rise has been slow. Though CR rate has been higher in *Klebsiella* compared to *E.Coli* the trend is very similar. CR rate in *Acinetobacter* remained stable but high over the last 5 yrs ranging between 75-85%. Recently there is a very encouraging trend of dropping resistance rate. *Pseudomonas* resistance rate also remained stable over the last few years with a very positive reduction in resistance over the last two years. Exact reason for the drop in CR in nonfermenters in our centre is not clear, but better environmental infection control and barrier precautions could be a reason.

Conclusion: Increasing carbapenem resistance in Enterobacteriaceae, though at a slower pace in the recent years, is a serious concern. There has been a decreasing trend in CR in nonfermentors particularly *Pseudomonas* over the last two years.

P1-GR21

Stability of colistin resistance in gram-negative bacterial strains

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Background: Colistin is considered to be the "drug of last resort" for the treatment of gram-negative MDR bacteria. Although still rare, colistin resistance has been observed in gram-negative pathogens. In this study, we investigated the stability of colistin resistance in four gram-negative pathogens.

Methods: Colistin-resistant mutants were obtained from 17 colistin-susceptible strains of four gram-negative species, *Acinetobaqcter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli,* by serial passage in culture containing progressively increasing concentrations of colistin. The stability of colistin resistance in these mutants was investigated by serial passage in colistin-free medium. Colistin susceptibility was determined by the broth microdilution method. Heteroresistance to colistin was also investigated in these bacterial strains.

Results: Exposure to sub-inhibitory concentrations of colistin caused a decrease in colistin sensitivity in all 17 isolates with high colistin MICs (≥64 mg/L). Three *P. aeruginosa* strains recovered colistin susceptibility; however, colistin-susceptible revertants were obtained from only one strain of *A. baumannii* and *E. coli* each. No susceptible revertants were obtained from *K. pneumoniae* mutants. Two *P. aeruginosa,* four *K. pneumoniae*, and three *A. baumannii* strains were heteroresistant to colistin.

Conclusion: Our study indicates that resistance to colistin in all four gram-negative species can be readily developed by repeated exposure to colistin. However, this colistin resistance was shown to be stable in three of the tested pathogenic species except *P. aeruginosa*.

P1-GR22

A distinct alleles and genetic recombination of *pmrCAB* operon in species of *Acinetobacter baumannii* complex isolates from Korea

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Objectives: To investigate *pmrCAB* sequence divergence in five species of *Acinetobacter baumannii* complex (or *A. calcoaceticus-A. baumannii* complex), a total of 80 isolates from bacteremia patients in a Korean hospital were explored in this study.

Methods: We determined complete sequences of *pmrCAB* operon for all isolates, and compared nucleotide and amino acid polymorphisms. Phylogenetic trees were constructed for each gene of *prmCAB* operon. Colistin and polymyxin B susceptibility was determined for all isolates and multilocus sequence typing was also performed for *A. baumannii* isolates.

Results: Our results showed that each species of *A. baumannii* complex have divergent *pmrCAB* operon sequences. We identified a distinct *pmrCAB* allele closely grouped with *A. nosocomialis* in gene trees. In addition, different grouping in each gene tree suggests sporadic recombination of *pmrCAB* genes among *Acinetobacter* species. Sequence polymorphisms among *Acinetobacter* species might not be associated with colistin resistance.

Conclusions: We revealed that a distinct *pmrCAB* allele may be widespread across the continents such as North America and Asia and that sporadic genetic recombination of *pmrCAB* genes might occur.

P1-GR23

AbaR-type resistance island in *Acinetobacter* species other than *A. baumannii*

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Objectives: In this study, we investigated the frequency of AbaR-type resistance island in *Acinetobacter* species other than *A. baumannii* isolates from Korea. In addition, the structure of their AbaR-type resistance islands was also determined.

Methods: A total of 155 isolates of nine non-*baumannii Acinetobacter* species were included in this study. To identify the presence of the AbaR-type resistance island, we tried to detect intact *comM* gene. If intact *comM* gene could not be obtained, regarded as presence of resistance island, to identify definite resistance island, PCR assay was performed using previously published primers. The structure of AbaR-type resistance island was determined by sequential PCR amplification and sequencing.

Results: Among 155 non-*A. baumannii* isolates, RIs were found to interrupt *comM* in five isolates: three *A. nosocomialis* and two *A. seifertii* (formerly *Acinetobacter* genomic species Close to 13TU). These structures were similar to those of AbaR-type RIs in *A. baumannii* isolates from Asian countries, including Korea, such as Tn*6167*-like, Tn*6167*-like lacking *tniD*, AbaR4, and simultaneous existence of Tn*6166* and AbaR3.

Conclusions: We identified AbaR-type resistance islands in non-*baumannii Acinetobacter* species isolates. They may be transferred from *A. baumannii.*

P1-GR24

Effect of combination of silver and copper II with colistin and imipenem against *Acinetobacter baumannii* isolates

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Background: Silver and copper have been used for antimicrobial properties for centuries. In this study, we tested whether theses

metals have synergistic effects to kill *Acinetobacter baumannii* with imipenem and colistin.
Methods: MICs of two antimicrobial agents (colistin and imipenem) were determined by a broth microdilution method for *A. baumanii* clinical isolates. Among those five *A. baumannii* isolates were selected having a wide range of MIC values (colistin, 0.5-64 mg/L; imipenem, 0.5-128 mg/L). Time-kill assays were performed using two metals sulfates (copper, 100 uM or 200 uM; silver, 0.1 uM) and two antimicrobial agents singly or in combinations at concentration of 1/4x MICs.
Results: The combination with copper II sulfate and imipenem showed synergistic effect against three imipenem-resistant *A. baumannii* isolates compared with single regimen of imipenem. However, no synergy effect was observed when copper II was combined with colistin. Silver plus colistin showed synergy effect along in three *A. baumannii* isolates, but re-growth was observed. The combination of sliver and imipenem showed similar results with the combination of silver and colistin.
Conclusion: In this study, we identified synergy effect of copper II sulfate with imipenem against some imipenem-resistant *A. baumannii* isolates.

P1-GR25
Complete sequence of a *bla*$_{KPC-2}$-harboring IncN2 plasmid from *Klebsiella pneumoniae* in Korea
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Objective: Emergence of plasmids harbouring *bla*$_{KPC-2}$ is a major public health concern due to their association with multidrug resistance and their potential mobility. In this study, we determined the complete sequence of a *bla*$_{KPC-2}$-harboring IncN2 plasmid of a *Klebsiella pneumoniae* isolate from Korea to investigate its emergence mechanism.
Methods: Antimicrobial susceptibility testing, multilocus sequence typing (MLST), and experiment on the conjugal transfer of plasmid were performed. IncN2-type plasmid pKPC-DK05 was extracted from a *K. pneumoniae* strain. Complete sequencing were done using the 454-Genome Sequencer FLX system. Contig assembly and gap closures were confirmed by PCR-based sequencing. Comparative genomic analyses of Tn4401b structure were performed to several other non-ST258 *bla*$_{KPC}$-carrying IncN plasmids.
Results: The transconjugant of the plasmid harboring *bla*$_{KPC-2}$ was resistant to carbapenems, cephalosporins, and piperacillin/tazobactam, but susceptible to tigecycline and polymyxin B. A plasmid pKPC-DK05 (56,294 bp) was very similar structure to IncN3 *Citrobacter freundii* pN-Cit backbone including replication gene (*repA*) and stability operons as well as transfer (*tra*) systems. *bla*$_{KPC-2}$ was encoded within a novel Tn4401b element containing *tnpR, tnpA*, IS*Kpn6*, and IS*Kpn7* (*istAB*). Comparative genomic analyses were characterized as a *bla*$_{KPC-2}$ mobilization element in several *bla*$_{KPC-2}$-producing isolates from the United States, Brazil, and Italy.
Conclusion: It is likely that the *bla*$_{KPC-2}$ plasmid is emerged by recombination among *K. pneumoniae* and other *Enterobacteriaceae* It would be a feature key to the spread and high prevalence of multidrug-resistant strains.

P1-GR26
Mutant prevention concentration (MPC) of tigecycline in *Acinetobacter baumannii* and *Klebsiella pneumoniae* clinical isolates
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Background: Although tigecycline is a promising therapeutic option for multidrug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* infections, tigecycline-non-susceptible isolates have been reported in patients receiving tigecycline.
Methods: To examine evolutionary mechanism of resistant mutants during clinical tigecycline dosing, mutant prevention concentrations (MPCs) of tigecycline were determined. And the effect of efflux pumps (AdeABC in *A. baumannii;* AcrAB-TolC in *K. pneumoniae*) and their local repressor (AdeRS in *A. baumannii;* AcrR in *K. pneumoniae*) on emergence of tigecycline resistant mutants were investigated.
Results: MPCs of tigecycline for *A. baumannii* and *K. pneumoniae* clinical isolates were ranged 1-4 mg/L and 4-16 mg/L, respectively. Amino acid alteration of AdeABC, AcrAB-TolC and AcrR was not identified in *A. buamannii* and *K. pneumoniae*, respectively. But, disruption of *adeS* by IS*aba1* was found in one *A. buamannii* isolates. Compared with parental isolates, the *adeB* and *acrA* expression increased 1-71.6 fold and 1.7-17.2 fold in in single-step mutants of *A. baumannii* and *K. pneumoniae*, respectively.
Conclusions: Since a recommended dose regimen of tigecycline is within the MSW, current clinical dosage regimen may be involved in the development of tigecycline-resistant mutants with up-regulation of efflux system.

P1-GR27
Mutant prevention concentration (MPC) of colistin alone and in combination with other antimicrobials
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Background: Current clinical dosage regimen of colistin may induce the occurrence of colistin-resistant mutants. It has been suggested that colistin can be used in combination with another class of antibiotic to prevent the evolution of colistin-resistant mutants.
Methods: Based on synergistic activity of colistin with ciprofloxacin, imipenem, rifampicin, and tetracycline against Gram-negative rods, we investigated the change in the MPCs of colistin alone and in combination in five each of *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Klebsiella. pneumoniae* clinical isolates. The broth microdilution method for MIC and a series of agar plates containing different concentrations of colistin were inoculated with 10^{10} colony-forming units of the bacterial culture for MPC were used.
Results: The MPCs of colistin were very high in the three Gram-negative rods (MPCs, 64 mg/L to >128 mg/L) when used alone. When colistin was combined with ciprofloxacin or imipenem, a dramatic change in the MPC was found only in ciprofloxacin or imipenem susceptible isolates, respectively. When colistin was combined with tetracycline, all isolates showed high colistin MPCs (≥64mg/L). Combination of rifampicin showed the most consistent effect in decreased of colistin MPC (16-32 mg/L).

Conclusions: In this study, the combination of colistin with other antimicrobial agents may have no significant effect in prevention of colistin resistance in Gram-negative pathogens, indicating that appropriate use of colistin may be the most important in prevention of colistin resistance.

P1-GR28

Decreased hypermucoviscosity and increased fitness cost in colistin-resistant *Klebsiella pneumoniae* ST23 strains

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Background: Highly invasive strains of *Klebsiella pneumoniae* ST23 strains that exhibit the hypermucoviscous (HV) phenotype have been reported as human pathogens, causing liver abscesses in patients.
Methods: Colistin-resistant mutants were developed *in vitro* from the three colistin-susceptible *K. pneumoniae* ST23 strains with HV phenotype. The lipid A structures of the three pairs of isogenic strains were analyzed by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. Changes in HV were investigated using the string test, and capsular polysaccharide (CPS) production was quantified. The expression levels of the *magA,* and p-*rmpA2* genes, serum resistance, biofilm-forming activity and fitness were compared between colistin-susceptible parental strains and colistin-resistant mutants.
Results: The addition of aminoarabinose or palmitate to the lipid A of lipopolysaccharide was examined in the colistin-resistant derivatives. The three colistin-resistant mutants exhibited reduced HV phenotype, decreased formation of CPS, and reduced expression of *magA* and p-*rmpA2*. In addition, survival rates in the presence of normal human serum, biofilm-forming activity, and fitness were decreased in the three mutant strains.
Conclusions: In hypervirulent HV *K. pneumoniae* ST23 strains, the acquisition of colistin resistance after stimulation with colistin was accompanied by reduced HV, reduced CPS production, impaired virulence, and a significant fitness cost.

P1-GR29

Emerging MDR *Acinetobacter baumanii* clusters in Hong Kong

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Multidrug resistant *Acinetobacter baumanii* (MDRA) is a significant nosocomial pathogen which is emerging globally. *A. baumannii* is intrinsically resistant to first-line antimicrobial agents, and has a tendency to develop resistance to second- and third-line agents. Numerous studies have reported the occurrence of multidrug-resistant (MDR) *A. baumannii* in hospitals and in Hong Kong. An alarming rise in MDR- and carbapenem-resistant *A. baumannii* in hospitals has been noted in recent years. The carbapenem resistance rate in *A. baumannii* rose from 1.1% in 2002 to 29.4% of all *A. baumannii* isolated in 2008 in Hong Kong. Institutional outbreaks caused by these MDR strains have constituted a growing public-health burden. We aim to study the relatedness of these MDRAs in Hong Kong.
MDRA strains, between June 2014 and January 2015, from different outbreaks, and one outbreak in 2011, were investigated to examine their relatedness. A total of 11 clusters comprising of 111 non-duplicate MDRAs were obtained from patients and environmental sampling from the hospitals within the New Territories East Cluster. Antibiotic susceptibilities of these strains were performed and interpreted according to CLSI (2014). A combination of randomly Amplified Polymorphic DNA (RAPD)–PCR typing, pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were used to create and compare fingerprints of the strains. The RAPD analysis revealed 2 predominant clusters (comprising of 73% of the organisms). Representative isolates from the 2 clusters were selected for typing by pulse field gel electrophoresis (PFGE), which showed that they were of the same pulsotype. These results reiterate the importance of control measures to prevent the transmission of clonal MDRA strains in nosocomial settings.

P1-GR30

Colistin resistance mechanism in *Acinetobacter nococomialis* and *Acinetobacter seiferti* isolates

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Background: Although high colistin resistance rates have been observed in *A. nosocomialis* and *A. seiferti* recently, colistin resistance mechanism is still unknown.
Materials and methods: Each six colistin-susceptible and colistin-resistant clinical isolates of *A. nosocomialis* and *A. seiferti* were included. Antimicrobial susceptibility testing and MALDI-TOF MS analysis with lipid A extraction were performed. To investigate an effect of efflux pump to colistin resistance in *A. nosocomialis* and *A. seiferti* isolates, colistin MICs were determined with combination of 10 μM carbonyl cyanide 3-chlorophenylhydrazone (CCCP), an efflux pump inhibitor. In addition, quantification of *pmrB* and *pmrC* gene expression was performed by qRT-PCR.
Results: In 12 isolates of two *Acinetobacter* species isolates, an addition of phosphoethanolamine (123 *m/z*) to the hepta-acylated lipid A (1910 *m/z*) was observed in some of colistin-susceptible and colistin-resistant isolates. An addition of HPO3 (80 m/z) was observed to two colistin-resistant isolates of *A. seiferti*. However, no specific peaks associated with colistin resistance were found in colistin-resistant *A. nosocomialis* and *A. seiferti* isolates, compared with colistin-susceptible isolates. The synergistic effect of CCCP was much greater in colistin-resistant isolates in both *A. nosocomialis* and *A. seiferti*. *pmrB* and *pmrC* expression levels did not show consistent results.
Conclusion: Unlike *A. baumannii*, lipid A modifications were not identified consistently in colistin-resistant *A. nosocomialis* and *A. seiferti* isolates. Instead, efflux pumps may be involved in colistin resistance in *A. nosocomialis* and *A. seiferti*.

P1-GR31

Efflux pumps involved in resistance to trimethoprim-sulfamethoxazole in *Stenotrophomonas maltophilia* isolates from Korea

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Background: *Stenotrophomonas maltophilia* is one of the opportunistic pathogens of growing clinical significance and is the third most common non-fermentative Gram-negative bacillus that has been isolated from clinical specimens. Trimethoprim-sulfamethoxazole (TMP/SMX) is the recognized antimicrobial of choice for the treatment of infections caused by *S. maltophilia*. However, resistance to TMP/SMX in *S. mlatophilia* has been increasingly reported worldwide. In this study, we aimed to investigate efflux pump systems involved in resistance to TMP/SMX in *S. maltophilia* isolates in Korea.
Methods: Clinical *S. maltophilia* isolates used in this study were collected from seven tertiary-care hospitals in Korea from 2007 to

2011. When minimum inhibitory concentrations (MICs) of TMP/SMX in TMP/SMX-resistant *S. maltophilia* strains were tested with efflux pump inhibitors (EPIs), EPIs decreased the TMP/SMX MICs in all resistant isolates. Expression of 13 efflux pump systems encoded in the *S. maltophilia* K279a genome were investigated and compared between TMP/SMX-resistant and TMP/SMX-susceptible *S. maltohilia* isolates using quantitative real-time PCR.

Results: Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) decreased the MICs of TMP/SXT by four- to 64-fold in TMP/SXT-resistant *S. maltophilia* isolates. Several efflux pump genes, for example *smeA*, *smeF*, and *smeK*, were consistently overexpressed in TMP/SXT-resistant isolates rather than susceptible isolates.

Conclusion: In this study, we showed that efflux pumps may play an important role in TMP/SMX resistance in *S. maltophilia*. We also identified several efflux pump systems involved in TMP/SMX resistance in *S. malto*philia.

P1-GR32

Proteomic analysis of outer membrane vesicles (OMVs) derived from antimicrobial resistant and sensitive *Escherichia coli*

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OMVs have been observed to be released from gram-negative bacteria. These OMVs help in defending cells against outer membrane-acting antibiotics based on the nearly identical surface ingredients of the OMVs and the bacterial outer membrane. In this way, they are suspected to be involved in antimicrobial resistance, thus antimicrobial sensitive bacteria would survive in antibiotic media by adding the OMVs from antimicrobial resistant bacteria. The OMVs from antimicrobial sensitive bacteria (RC85) or antimicrobial resistant bacteria (RC85+) were purified by ultracentrifugation. The morphology of their OMVs was monitored by transmission electron microscopy. To evaluate the effects of the OMVs, the growth rates of RC85 treated with the OMVs from RC85 or RC85+ were measured. The OMVs from RC85 or RC85+ were analyzed using LC-ESI-MS/MS to compare their protein compositions. As a result of this study, we found that the OMVs from RC85+ diminished the activity of the antibiotics to inhibit the growth rate of RC85, thus let RC85 keep growing. From the result of the protein analysis, 103 and 163 proteins were uniquely found in OMVs from RC85 and RC85+, respectively. The OMVs from RC85 solely possessed chain O and I proteins, and OMVs from RC85+ possessed long-chain-fatty-acid-CoA ligase. In this study, we demonstrated that the survival rate of RC85 in the antibiotic media was improved with the treatment of the purified OMVs from RC85+. Furthermore, we compared the protein compositions of the OMVs from RC85 or RC85+ to evaluate the proteomes involved in the antimicrobial resistance. With the information, we suggest that the presence of these proteins found in the OMVs from RC85+ or RC85 is essential for the bacterial growth and survival in an environment with antibiotics.

P1-GR33

Effect of ascorbic acid on colistin-associated nephrotoxicity: a preliminary clinical study

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Background: Nephrotoxicity is a dose-limiting factor of colistin, a last-line therapy for multidrug-resistant Gram-negative bacterial infections. An earlier animal study revealed a protective effect of ascorbic acid against colistin-induced nephrotoxicity. The objective of the study was to determine the potential nephroprotective effect of ascorbic acid against colistin-associated nephrotoxicity in patients receiving intravenous colistimethate sodium (CMS) for treatment of carbapenem-resistant (CR) Gram-negative bacterial infections.

Methods: Randomized controlled study was conducted in patients with CR Gram-negative bacterial infections who received CMS. Ascorbic acid was administered intravenously at a dose of 2 grams every 12 h in the patients randomized to CMS-ascorbic acid group. CMS was given for 7-14 days and ascorbic acid was discontinued at the same time as CMS. Nephrotoxicity was defined by RIFLE classification system. Urinary neutrophil gelatinase-associated lipocalin (NGAL) and *N*-acetyl-beta-D-glucosaminidase (NAG) were measured as markers of renal damage, and plasma concentrations of formed colistin were quantified.

Results: An interim analysis in 13 patients who received CMS plus ascorbic acid and 15 who received CMS alone revealed that the incidence of nephrotoxicity was 53.8% and 60.0% in the CMS-ascorbic acid group and the CMS group, respectively (p 0.96). In both groups, urinary excretion rates of NGAL and NAG after CMS treatment were significantly higher than those at the baseline ($p<0.05$). However, urinary excretion rates of these biomarkers at the various times during CMS treatment did not differ significantly between the groups ($p>0.05$). Plasma concentrations of formed colistin in both groups were not significantly different ($p>0.28$). The clinical and microbiological outcomes and mortality of the patients in both groups were not significantly different.

Conclusion: This preliminary study suggests ascorbic acid does not offer nephroprotective effect for patients receiving intravenous CMS.

P1-GR34

Predictive value of NA resistance for increased minimum inhibitory concentration (MIC) of fluoroquinolone in clinical isolates of *Salmonella enterica*.

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Introduction: Resistance in Salmonella enterica to Chloramphenicol, Amoxycillin and Co-trimoxazole has posed a challenge to treatment of typhoid fever. Ciprofloxacin has been the empirical therapy of choice, but the recent increase in minimum inhibitory concentration (MIC) to ciprofloxacin in Salmonella enterica, not detectable by disc diffusion (DD) tests, may result in delayed response and serious complications. Nalidixic acid (NA) resistance has been used as an indirect evidence of increased Ciprofloxacin MIC in Salmonella enterica. This study evaluated the predictive value of NA resistance for increased MIC of fluoroquinolone in clinical isolates of Salmonella enterica.

Materials and methods: A total of 51 clinical isolates of *Salmonella enterica* serovars Typhi and Paratyphi A were tested for antimicrobial susceptibility test, by modified Kirby Bauer disc diffusion method as per the guidelines of the Clinical Laboratory Standards Institute(CLSI). MIC determination of Ciprofloxacin was performed by agar dilution method.

Results: Out of 51 *Salmonella enteric* isolates, 20 (39.2%) isolates were *S. Typhi* and 31 (60.8%) were *S. Paratyphi A*. All the isolates were sensitive to Ceftriaxone. Only 4 isolates were MDR. Forty-eight isolates were resistant to NA. MIC of Ciprofloxacin toward NA resistant isolates were found to be >8μg/ml that towards NA sensitive and intermediately sensitive isolates were found to be < 2μg/ml.

Conclusions: Disc diffusion test failed to detect the reduced susceptibility of Ciprofloxacin. Hence, the MIC determination of Ciprofloxacin against *Salmonella enterica* isolates would be important. Nalidixic acid (NA) resistance can be used as an indirect evidence of increased Ciprofloxacin MIC in *Salmonella enterica.*

P1-GR35
Genotypes of ciprofloxacin-resistant *Klebsiella pneumoniae* in Korea and their characteristics according to the genetic lineages

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Objectives: To investigate the resistance mechanism and molecular epidemiology of ciprofloxacin-resistant *Klebsiella pneumoniae* from Korea.
Methods: For 160 *K. pneumoniae* collected in 2013, ciprofloxacin MICs were determined by agar dilution method. To characterize the genotypes of ciprofloxacin-resistant *K. pneumoniae* isolates, multilocus sequence typing (MLST) and *wzi* gene typing were performed. The presence of plasmid-mediated resistance determinants (*qnrA*, *qnrB*, *qnrS*, *aac(6')-Ib-cr*, $bla_{\text{CTX-M}}$ and bla_{SHV}) were investigated. *GyrA* and *parC* genes were sequenced.
Results: Fifty-seven isolates showed ciprofloxacin resistance and *qnr* and *aac(6')-Ib-cr* was carried by 50 (87.7%) and 32 (56.1%) isolates, respectively (p=0.0002). By MLST, 20 STs were identified. The two most prevalent clones were ST307 (14/57, 24.6%) and ST11 (12/57, 21.1%). All the ST307 isolates harboured *qnrB* and *aac(6')-Ib-cr* and $bla_{\text{CTX-M-1 group}}$ was carried by 12 and 13 isolates, respectively. Among the 12 ST11 isolates, *qnrB*, *qnrS* and *aac(6')-Ib-cr* was carried by 9, 7, and 3 isolates, respectively and SHV-type ESBL and $bla_{\text{CTX-M-1 group}}$ was carried by 9 and 4 isolates, respectively. The mutation in the *gyrA* and *parC* was found in 50 and 48 isolates, respectively and the majority harbored *gyrA* (S83I) and *parC* (S80I). By *wzi* gene sequencing, 49 isolates could be differentiated. All the 14 ST 307 isolates showed identical sequence but ST11 isolates showed various *wzi* gene sequence type.
Conclusion: *K. pneumoniae* ST307 and ST11 were the two predominant clones and the distribution of the plasmid-mediated resistance determinants was different between two clones. The *wzi* gene sequencing could be a useful tool for identifying ST307 isolates.

P1-GR36
Trends in ESBL-production and susceptibility for *E. coli* from urinary tract infections in Southeast Asia: SMART 2010-2013

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Background: The Study for Monitoring Antimicrobial Resistance Trends (SMART) has monitored the *in vitro* susceptibility of gram-negative organisms from urinary tract infections (UTI) since late 2009. This report summarizes trends in extended-spectrum β-lactamase (ESBL) production and susceptibility of *Escherichia coli* from UTI from 2010 to 2013 in Southeast Asia.
Methods: 1,327 *E. coli* were collected from UTI by 12 hospitals in 5 countries. MICs and ESBL phenotypes were determined by CLSI broth microdilution, and interpreted using CLSI guidelines. Linear trends in ESBL+ rate and susceptibility were evaluated using the Cochran-Armitage test.
Results: Trends in % ESBL+ *E. coli* are shown in Figure 1. Although there was an increasing trend in ESBL+ *E. coli* in Southeast Asia, it did not reach statistical significance (p=0.08). A sensitivity analysis using only the 10 sites that participated in all 4 years showed a similar but slightly clearer trend (34%, 40%, 41%, and 42% in 2010-2013, p=0.06). Susceptibility in Southeast Asia was low for cephalosporins (generally ~60% or less) and fluoroquinolones (<45%). Only amikacin, ertapenem, imipenem, and piperacillin-tazobactam demonstrated susceptibility ~90% or higher.
Conclusion: ESBL+ *E. coli* levels varied by country, with especially high rates in Vietnam. An increase from 2010 to 2011 was observed for Southeast Asia overall, with rates ~40% during 2011-2013. As expected considering the high ESBL rates and co-resistance often found in ESBL+ isolates, susceptibility was low for cephalosporins and fluoroquinolones, limiting therapeutic options for UTI in Southeast Asia.

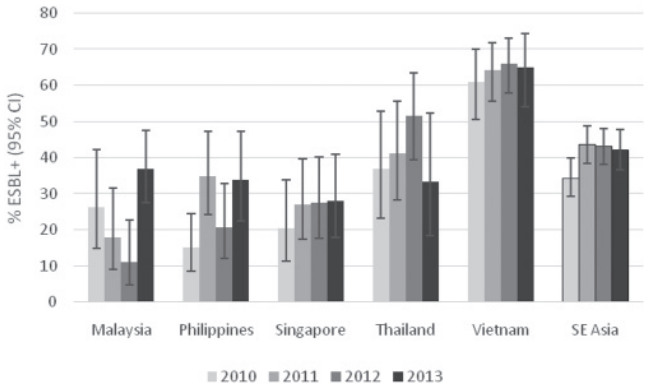

Fig. 1.

P1-GR37
Variability of susceptibility and multidrug resistance among *K. pneumoniae* from IAI in Asia/Pacific countries – SMART 2012-2013.

D. Biedenbach, S. Lob, R. Badal, D. Sahm. *International Health Management Associates, Inc., USA*

Background: The Study for Monitoring Antimicrobial Resistance Trends (SMART) has monitored the *in vitro* susceptibility of gram-negative organisms from intra-abdominal infections (IAI) since 2002. This report documents the variable susceptibility and multidrug resistant (MDR) percentages of *K. pneumoniae* from IAI during 2012 and 2013 in Asia/Pacific (APAC) countries.
Methods: 1,864 *K. pneumoniae* were collected from patients with IAI in 11 countries. MICs were determined by broth microdilution, and interpreted using CLSI guidelines. ESBL rates are determined using phenotypic screen-positive results to third-generation cephalosporins per CLSI.
Results: Overall susceptibility of *K. pneumoniae* phenotypes are shown in Table 1.

Table 1.

	All *K. pneumoniae* (1,864)		ESBL + (453)		MDR (315)	
Drug	% Susc.	MIC_{90}	% Susc.	MIC_{90}	% Susc.	MIC_{90}
Amikacin	95.4	<= 4	85.2	> 32	74.0	> 32
Cefepime	79.4	> 32	20.3	> 32	25.7	> 32
Ceftazidime	77.4	64	26.9	> 128	11.4	> 128
Ceftriaxone	70.0	> 32	2.2	> 32	3.2	> 32
Ciprofloxacin	76.7	> 2	34.9	> 2	7.6	> 2
Ertapenem	92.7	0.5	81.2	> 4	61.3	> 4
Imipenem	95.1	1	87.2	4	74.0	> 8
Levofloxacin	83.4	> 4	54.8	> 4	24.4	> 4
Pip/tazobactam	88.3	32	66.0	> 64	48.6	> 64

MDR defined as resistant to 3 or more drug classes

ESBL rates (n tested) were 40% (929) in China, 29% (73) Thailand, 27% (131) Malaysia, 26% (160) Singapore, 23% (164) Philippines, 22% (119) Vietnam, 20% (116) New Zealand, 13% (120) South Korea, 9% (165) Australia, 7% (109) Hong Kong, and 6% (63) in Japan and (451) Taiwan. MDR rates were highest in Philippines and China (26%) and lowest in Australia (3.5%).

Conclusions: Within the APAC region, variability between countries of ESBL and MDR rates was observed among *K. pneumoniae*. This species continues to be a common IAI pathogen capable of carrying multiple resistance mechanisms that target β-lactams, fluoroquinolones, and aminoglycosides. MDR *K. pneumoniae* isolates need ongoing surveillance as drug resistance to this species continues to increase.

P1-GR38

Antimicrobial resistance in *Enterobacteriaceae* from intra-abdominal infections in ICU and non-ICU wards in Asia/Pacific: SMART 2012-2013

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Objectives: Increased resistance has been reported in *Enterobacteriaceae* from intensive care units (ICUs), limiting treatment options in this setting. Using Asia/Pacific data from the Study for Monitoring Antimicrobial Resistance Trends (SMART), susceptibility of *Enterobacteriaceae* from intra-abdominal infections (IAI) was analyzed according to patient location.

Methods: 6,730 *Enterobacteriaceae* isolates from IAI were collected in 11 Asia/Pacific countries and Hong Kong in 2012-2013. Only countries with isolates from both ICU and non-ICU wards were included. MICs were determined by CLSI broth microdilution method and interpreted using CLSI guidelines. Isolates were categorized as multi-drug resistant (MDR) if resistant to ≥3 of the tested drug classes.

Results: The distribution of species was similar in ICU and non-ICU wards, with prevalence of 52.3 and 53.7%, respectively, for *E. coli*; 26.6 and 23.5% for *K. pneumoniae;* and 6.5% and 6.0% for *E. cloacae*. Susceptibility (%S) of *Enterobacteriaceae* to a subset of agents and % MDR are shown.

	ICU	non-ICU
% S Ertapenem*	90.3	94.8
% S Imipenem*	88.2	91.9
% S Cefepime*	64.8	73.9
% S Ceftriaxone*	49.8	58.9
% S Pip-tazo*	82.6	89.6
% S Levofloxacin	65.7	68.9
% S Amikacin*	94.3	97.0
% MDR*	27.5	21.6
n	864	5866

* Significant difference between ICU and non-ICU (p<0.05, chi-square test).

% MDR were also significantly different between ICU and non-ICU wards for *E. coli* (32.3 vs. 26.3%, respectively) and *K. pneumoniae* (25.2 vs. 16.6%).

Conclusions: *Enterobacteriaceae* isolates from ICUs were significantly less susceptible than those from non-ICU wards to most agents and showed a higher % MDR. Of the tested agents, only ertapenem and amikacin maintained susceptibility >90% against *Enterobacteriaceae* in both settings. Knowledge of resistance patterns across hospital settings is crucial both for infection control efforts and treatment decisions.

P1-GR39

Distribution of NDM-producing *Enterobacteriaceae* in Asia and impact on phenotypic detection of ESBLs

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Background: *Enterobacteriaceae* co-producing carbapenemases (CRE) and other β β-lactamases complicate the use of standard clavulanate-based phenotypic ESBL tests because of their differential sensitivities to β β-lactamase inhibitors (BLIs). Asian countries have significant ESBL rates; however NDM-producing isolates are becoming more common in this region. We compared phenotypic and molecular ESBL detection methods for NDM-producing isolates.

Methods: MICs were determined by CLSI broth microdilution (BMD). Isolates with off-scale MIC values to ceftazidime and cefotaxime +/- clavulanate were further examined by disk approximation (DA) and screened for β-lactamase genes by PCR and sequencing. 97 NDM-positive isolates were identified in a surveillance collection of *Enterobacteriaceae* submitted from Asia in 2008-2013.

Results: Detected β-lactamase combinations are listed in Table 1.

Table 1.

β-lactamase (n)	Countries (n)*
NDM only (8)	IN (2), PH (2), TH (1), VN (3)
NDM + AmpC (9)	
CMY (7)	IN (3), VN (4)
DHA (1)	IN (1)
CMY + DHA (1)	VN (1)
NDM + ESBL (39)	
CTX-M-15 (30)	IN (17), PH (8), MY (1), VN (4)
CTX-M-14 (1)	VN (1)
CTX-M-27 (1)	PH (1)
SHV-12 (1)	PH (1)
SHV-28 (1)	VN (1)
CTX-M-15 + SHV-12 (4)	IN (3), PH (1)
CTX-M-15 + SHV-2 (1)	IN (1)
NDM + ESBL + AmpC (41)	
CTX-M-15 + CMY (29)	IN (26), VN (3)
CTX-M-15 + DHA (4)	IN (3), PH (1)
CTX-M-14 + ACT/MIR (1)	VN (1)
CTX-M-27 + CMY (2)	VN (2)
SHV-2 + DHA (1)	IN (1)
CTX-M-15 + CMY + DHA (4)	IN (2), VN (2)

* India (IN), Malaysia (MY), Philippines (PL), Thailand (TH), Vietnam (VN)

NDM-producing CRE (92.8% NDM-1) included 54 *K. pneumoniae*, 42 *E. coli*, and 1 *K. oxytoca*. All were ESBL non-determinable by BMD and 91 were ESBL- by DA, even though 80 (82.5%) carried ESBLs based on molecular characterization.

Conclusions: Carbapenemases, including metallo-β-lactamases, are becoming increasingly common worldwide. NDMs can cause false-negative or inconclusive phenotypic ESBL tests; it is therefore increasingly important to categorize pathogens by carbapenemase status as well as their ESBL status.

P1-GR40

Determination of gram negative isolates and their antibiogram from different clinical samples at a tertiary care hospital, Kathmandu

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Background: The emergence and spread of antimicrobial resistance constitutes a major risk for human health by limiting

the success of these agents in the therapy and contributing to morbidity and mortality. The most probable reason is the widespread use of antibiotics and often choosing an inappropriate drug. Intrinsic factors and extrinsic factors have been postulated for the development of resistance. Prudent and rational use of antimicrobial is possible by forming local, national and global wide antibiogram profile.

Methods: A retrospective study was conducted at Tertiary Care Hospital, from April 14th to 17th September 2014. Data were collected from hospital registration books and analyzed using SPSS version 20 software.

Results: Klebsiella spps were the major predominant. Most of the isolates were MDR except Shigella spps which was only resistant to Amoxicillin (100%) and Proteus which was only resistant to Erythromycin (100%). Isolates showed resistant to major group of antibiotics but high level resistance, High resistance to 3rd Generation Cephalosporin's was the marker for the presence of ESBL. Brief summary is given in Table 1.

Table 1. Resistance pattern of gram negative organisms

Antibiotics	Kleb-siella Spps	Acineto-bacter Spps	E. coli Spps	Proteus spps	Pseudo-monas Spps	Shigella Spps
Amikacin	38.3%	50.0%	13.6%	0.0%	0.0%	0.0%
Gentamicin	25.0%	66.7%	0.0%	0.0%	0.0%	0.0%
Tobramycin	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%
Ceftriaxone	71.4%	72.2%	67.4%	0.0%	30.0%	0.0%
Ceftazidime	100.0%	100.0%	100%	0.0%	100.0%	0.0%
Cephotaxime	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%
Nalidixic acid	0.0%	100.0%	50.0%	0.0%	0.0%	0.0%
Ofloxacin	58.3%	100.0%	20.0%	0.0%	0.0%	0.0%
Ciprofloxacin	61.5%	58.8%	72.5%	0.0%	25.0%	0.0%
Norfloxacin	33.3%	0.0%	100.0%	0.0%	0.0%	0.0%
Erythromycin	93.5%	75.0%	92.0%	100.0%	100.0%	0.0%
Azithromycin	33.3%	100.0%	17.6%	0.0%	0.0%	0.0%
Amoxicillin	100.0%	90.0%	96.9%	0.0%	100.0%	100.0%
Piperacillin/Tazobactam	33.3%	33.3%	0.0%	0.0%	100.0%	0.0%
Chloramphenicol	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Cotrimoxazole	68.4%	87.5%	66.7%	0.0%	88.9%	0.0%
Nitrofurantoin	50.0%	100.0%	0.0%	0.0%	0.0%	0.0%
Tetracycline	25.5%	25.0%	38.1%	0.0%	87.5%	0.0%
Novobiosin	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Imipenim	6.2%	28.6%	0.0%	0.0%	33.3%	0.0%
Cefepime	100.0%	0.0%	100.0%	0.0%	0.0%	0.0%

Conclusion: The results of this study demonstrated an alarming prevalence of resistant organism. Appropriate and judicious selection of antibiotic by using antibiogram profile would limit the emerging drug resistant strains. Detail study is required with regard to proper anti biotic usage, susceptibility testing and prescribing policies.

P1-GR41

Emergence of multidrug-resistant *Acinetobacter baumannii* in a tertiary care hospital, Mongolia

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Although Multidrug-resistant (MDR) *Acinetobacter baumannii* has emerged in recent years as a leading cause of nosocomial infections in many countries, there are a few reports from Mongolia. A total of 169 *A. baumannii* isolates were recorded between January 2013 and February 2014 from the First Central hospital in Mongolia. The resistance rate to ceftazidime, cefepime, piperacillin/tazobactam and ciprofloxacin was between 82.1-89.1 %, followed by imipenem and amikacin (40.8 %-52.5 %) in ICU. On the contrary, resistance to ceftazidime, cefepime, ciprofloxacin and piperacillin/tazobactam were between 31.4% and 48.3% in non-ICU areas. Resistance to amikacin and imipenem were 27.5% and 20%, respectively. A total of 35 *A. baumannii* isolates were subjected to additional testing. The results indicate that 52.3%, isolates belonged to ST195 (19/35), 42.0% isolates were ST642 (14/35), and 5.7% isolates were ST231 (2/35). Moreover, results showed highly similar PFGE types within the same ST-type and clonal spread of CPAB ST195 and ST642 (33/35, 94.3%). All isolates were susceptible to colistin and tigecycline. All ST195 isolates carried both $bla_{OXA\text{-}23\text{-}like}$ and $bla_{OXA\text{-}51\text{-}like}$ genes. Thirteen ST642 isolates (13/14) carried both $bla_{OXA\text{-}58\text{-}like}$ and $bla_{OXA\text{-}51\text{-}like}$, and 1 ST642 isolate (1/14) harbored only $bla_{OXA\text{-}51\text{-}like}$ gene. Moreover, 2 ST231 isolates had only the $bla_{OXA\text{-}51\text{-}like}$ gene. No bla_{VIM}-, bla_{IMP}-, bla_{SIM}-, bla_{NDM}-, and bla_{KPC}-producers were found in this study. In conclusion, we emphasize that high resistance rate and clonal spread of MDR *A. baumannii* of a Mongolian tertiary care hospital. A surveillance program in Mongolian hospitals is necessary in order to implement rapid health control policies.

P1-GR42

Ertapenem resistance by porin loss or alteration combined with CTX-M type ESBL production in carbapenemase-negative *Escherichia coli* clinical isolates from Thailand

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Background: The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) is of clinical concern for healthcare in many countries. In Thailand, carbapenemase-producing CRE isolates have been reported increasingly, whereas information of other resistance mechanisms is limited. Therefore, we characterized carbapenemase-negative ertapenem-resistant *Escherichia coli* (CNEREc) isolates collected between 2010 and 2014 from a Thai university hospital.

Methods: Eight non-repetitive CNEREc isolates with ertapenem MICs of >1 μg/ml were detected for extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase (AmpC) production by phenotypic tests and PCR methods. The *ompC* and *ompF* were detected by PCR and nucleotide sequencing. Clonal relatedness was performed by ERIC-PCR.

Results: The ertapenem, imipenem and meropenem MIC ranges for the 8 isolates were 2-32, 0.125-8 and 0.125-4 μg/mL, respectively. Seven isolates produced CTX-M type ESBLs, whereas no AmpC was found. All isolates exhibited the *ompF* and two isolates did not contain the *ompC*. Alterations of the *ompF* included mutations, 3-bp or IS*1* insertion, and/or 1-, 4-, 7- or 18-bp deletion, while those of the *ompC* were mutations and/or 1-, 9- or 12-bp deletion. Three different ERIC-PCR patterns were seen among the 8 isolates. In addition, closely related isolates from different patients showed different porin loss or alterations.

Conclusion: To our knowledge, this is the first report of ertapenem-resistant *E. coli* with porin loss/alteration combined with CTX-M ESBLs from Thailand.

P1-GR43

Phenotypic and genotypic characterization of carbapenem-resistant Enterobacteriaceae isolates from the Philippine antimicrobial resistance surveillance program (arsp)

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Background: The recent local emergence of resistance of Enterobacteriaceae to carbapenems makes management of both community and healthcare-associated infections problematic. These are often associated with a variety of typical antibiotic susceptibility profiles necessitating reliable local data that can provide evidence for empiric treatment recommendations.
Methods: This is a prevalence survey on carbapenem-resistant Enterobacteriaceae isolated from patients in ARSP sentinel sites from January 1, 2012 until December 31, 2013. All identified carbapenem-resistant Enterobacteriaceae isolates from clinical cultures tested by disk diffusion or MIC in the regional laboratories were sent to the reference laboratory for confirmation phenotypically for carbapenemase production by the modified Hodge test, and screened and tested for carbapenemase genotype by multiplex PCR for the genes KPC, IMP, OXA-48, VIM and NDM-1.
Results: A total of 174 isolates were confirmed at the reference laboratory. Genotyping by PCR revealed that the most common carbapenemase gene identified was the New Delhi beta-lactamase (NDM-1). Isolated KPC, IMP and OXA-48 genes were also identified. These CRE isolates also tested as resistant to most of the antibiotic classes (penicillins, cephalosporins, fluoroquinolones, co-trimoxazole and nitrofurantoin) but remained variably susceptible *in vitro* to the aminoglycosides.
Conclusion: The most commonly identified resistance mechanism for carbapenem-resistant Enterobacteriaceae in this study was the NDM-1 gene. Resistance patterns suggest poor susceptibility to most classes of locally available antimicrobials. This data supports the need for continuous surveillance for a national antibiotic stewardship program in the Philippines.

P1-GR44

Characterization and comparison of biofilm development by pathogenic and commensal isolates of *Pseudomonas aeruginosa* from hospitalized patients

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Pseudomonas aeruginosa is gram-negative coccobacillus that is ubiquitous in nature, persistent in the hospital environment and causes a variety of opportunistic nosocomial infections. The survival properties of *P. aeruginosa* most likely play a significant role in the outbreaks caused by this pathogen. The potential ability of *P. aeruginosa* to form biofilms could explain its outstanding antibiotic resistance and survival properties. In this study, biofilm forming potentials of various *P. aeruginosa* clinical strains were characterized by confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM) and tissue culture plates stained with crystal violet. There was a significant difference in their biofilm-forming ability among all 70 *P. aeruginosa* clinical strains. At 24 h of incubation, almost the entire surface area was green and the oblique-view CLSM image suggests that a thick biofilm was formed. The biofilm grown on glass for 24 h was observed using SEM, the matrix of the biofilm, formed a complex 3D structure, further irregularly shaped spaces resembling water channels also been observed among dense structures. Understanding the mechanism by which biofilms form, as well as characterizing their matrix components, is an area of intense interest. Our study provides a detailed analysis of biofilm formation potential in *P. aeruginosa*, which is a step towards understanding its role in pathogenesis and eventually lead to a better understanding of how to eradicate *P. aeruginosa* growing as biofilms with antibiotic therapy.
Keywords: *Pseudomonas aeruginosa*; Biofilm; Crystal violet assay; Confocal Laser Scanning Microscopy; Scanning Electron Microscopy; CRA

P1-GR45

Interplay of bla_{NDM-1} and MexAB-OprM transcriptional expression in *Pseudomonas aeruginosa* with single dose carbapenem exposure

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Background: Therapeutic option of carbapenem antibiotic is compromised in *Pseudomonas aeruginosa* due to expression of both acquired and intrinsic resistance mechanism. In recent years, New Delhi metallo beta-lactamase is in focus as predominant carbapenem resistance determinant. However, it is in question which mechanism responds in *P. aeruginosa* carrying bla_{NDM-1} and overexpressed MexAB-OprM system during carbapenem therapy. The study investigates interplay of both the mechanisms in clinical isolates of *P. aeruginosa* when exposed to meropenem.
Methods: Seven *P.aeruginosa* isolates with differing carbapenem MIC value, harbouring NDM-1 along with over expressed MexAB-OprM were incubated in L B broth with and without meropenem. At each 45 min interval total RNA was isolated, immediately reverse transcribed in to cDNA. Quantitative real time PCR was performed for both the resistance mechanisms. The RQ values obtained at different time intervals were compared with an isolate with over expressed MexAB-OprM system and constructing a bla_{NDM-1} clone in *P. aeruginosa* K2733 - PAO1 ΔMexAB-OprM ΔMexCD-OprJ ΔMexEF-OprN ΔMexXY-OprM.
Result: It was observed that meropenem exposure does not produce any significant elevation of transcriptional expression in either of the mechanism. However, it was an interesting finding that upon exposure to carbapenem, efflux pump system plays a major role in bacterial survival compared to NDM-1.
Conclusion: The study gives an insight in to bacterial response to carbapenem antibiotic when two different resistance mechanisms exist. This type of study is helpful in designing future antimicrobials which can take care of all the known resistance determinants and their interplay to offer an efficient treatment option.

P1-GR46

Carriage of multidrug resistant integron gene cassette arrays within environment and food isolates in a high altitude city of northeast India

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Background: Class 1 integrons are the primary source and reservoir of antimicrobial resistance gene, playing an important role in the dissemination of resistance genes among *Escherichia*

coli. The aim of this study was to investigate the genetic context and structural diversity of class 1 integron gene cassettes and their role in imparting multidrug resistance among *E. coli* isolated from environmental and food samples.
Methods: A total of 148 *E. coli* were isolated from water bodies (rivers, lakes, drains) and ready to eat food samples collected from different sites of north eastern India. Isolates were tested for the production of ESBLs, AmpC and carbapenemases. Presence of class 1 and 2 integrons and gene cassettes was analyzed by integrase and 59 base element PCR respectively. Further the amplified products were sequenced.
Results: The prevalence of class 1 integrons was 35% (52/148) which includes 28.7% (19/66) of water and 40.2% (33/82) of food borne *E. coli.* Six diverse types of genetic arrangement of integron were obtained, with *dfr*, *aadA* and *qacE* genes to be commonly present in all types. In addition, $bla_{OXA-2,}$ $bla_{CTX-M-15,}$ multidrug efflux protein, *aacA7* and *tetA* genes were also detected. All the six diverse integron were conferring resistant phenotype. The gene cassettes were disseminated among *E. coli* of diverse origin.
Conclusion: Our study highlights the potential public health risk of *E. coli* isolates carried in food samples and water sources as a reservoir of MDR cassettes. The dissemination of resistant islands seem to be enriched among water and food *E.coli* thus representing a vehicle for the acquisition and dissemination of antimicrobial resistance in this ecosystem. The reasons for this enrichment are unknown but a better knowledge of the routes of integron transference among bacteria could get insights to this problem.

P1-GR47
Antibiogram of Salmonella in enteric fever capital of the world - then and now: 'the dust hasn't settled yet'
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Background: Enteric fever remains an important public health problem in many countries of the world. Nepal-endemic of enteric fever is also known as 'enteric fever capital of world'. The development of antibiotic resistance in *Salmonella* poses considerable threat of increased mortality and morbidity to many communities of the world.
Method: A retrospective study was carried out at the microbiology department of DH-KUH. The prevalence of *Salmonella* and their antibiogram during the year 2004-05 were compared to that of 2012-13. The hospital has been using WHO recommended techniques for blood culture and organism identification. Susceptibility testing was done by Kirby and Bauer disk diffusion method (Hi media antibiotic disc) using CLSI guideline.
Result: A total of 7510 blood cultures (2170 from 2004/05 and 5340 from 2012/13) ascertained 437 Salmonella species. *Salmonella* isolation rate among blood cultures was found to be more than two times higher during 2004/05 as compared to that of 2012/13 (10.1% Vs 4.0%). *S.* Typhi was the most common isolate followed by *S.* Paratyphi A. No *S.* Paratyphi B was isolated during 2004/05 whereas 10.2% of *Salmonella* in 2012/13 were Paratyphi B. The NARS strain among isolated *Salmonella* reached 94.2% in 2012/13 from 41.4% in 2004/05. Only 8% of the organisms tested for ciprofloxacin were reported sensitive in 2012/13 against 90% in 2004/05. Effectiveness of Ampicillin was also considerably reduced in those 7 years. Differences in susceptibility to the Chloramphenicol, Co-trimoxazole and Ceftriaxone were not statistically significant.
Conclusion: The switching of Salmonella against our concurred antibiotics like fluroquinolones clearly displays a circumspect selection of antibiotic to treat enteric fever. The molecular basis for the justification of resistance is integral.

P1-GR48
Modulation of cytokine levels by kaempferol in a murine model of *Burkholderia pseudomallei* infection involved inhibition of glycogen synthase kinase-3β (GSK3β)
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Kaempferol, a natural flavonoid commonly present in medicinal plants, is reported to have many health benefits including anti-inflammatory and immunomodulatory properties. Kaempferol has also been shown elsewhere to activate the PI3K/Akt signaling pathway. Glycogen synthase kinase-3β (GSK3β), a critical downstream component of this pathway, is a serine/threonine kinase established to play a pivotal role in the regulation of host inflammatory response to infections by pathogens including *B. pseudomallei* through modulation of pro- and anti-inflammatory cytokines production. In this study, we investigate the involvement of GSK3β in the immunomodulatory effect of kaempferol in *B. pseudomallei* infection. Effects of kaempferol on survivability of mice experimentally-infected with *B. pseudomallei*, bacterial loads, phosphorylation states of GSK3β and levels of inflammatory cytokines (TNF-α, IFN-γ and IL-10) in liver, spleen and serum of infected mice were determined using a murine *B. pseudomallei* infection model. Our results revealed that intraperitoneal administration of kaempferol (10 mg/kg b.w.) at one day pre-infection significantly ($P<0.05$) improved survivability (33%) of *B. pseudomallei*-infected mice compared to non-treated infected controls. Bacterial loads in liver and spleen obtained from *B. pseudomallei*-infected mice treated with kaempferol showed no significant difference compared to non-treated infected controls. Administration of kaempferol resulted in significant ($P<0.05$) increase in phosphorylated GSK3β (pGSK3β) levels in both liver and spleen of *B. pseudomallei*-infected mice compared to non-treated infected controls. Levels of pro-inflammatory cytokines (TNF-α and IFN-γ) in liver and serum were significantly lowered by kaempferol administration into *B. pseudomallei*-infected mice compared to non-treated infected animals. The levels of anti-inflammatory cytokine (IL-10) were however significantly ($P<0.05$) raised in serum of kaempferol-administered infected mice. In conclusion, the observed immunomodulatory effects of kaempferol via maintaining a balanced-profile of pro- and anti-inflammatory cytokines are mediated through inhibition of GSK3β. Our findings provide scientific evidence that kaempferol is potentially beneficial as an anti-infective against *B. pseudomallei* infection.

P1-GR49
Association of biofilm production with colonization and infection among clinical isolates of *Acinetobacter baumannii*
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Background: *Acinetobacter baumannii* is important pathogen in healthcare-associated infection worldwide especially in intensive care unit. Biofilm formation, a virulence factor of *A. baumannii*, is associated with long-term survival in hospital environments and provides resistance to antibiotics. The present study aimed to identify the clinical impact of biofilm production in colonization and acquisition after admission.
Method: A total 49 *A. baumannii* isolates were obtained between August and November 2013 from Dongsan medical center, Daegu, Korea. All isolates were originated from sputum which were all new patients infected or colonized by *A. baumannii*. The microtiter plate assay was used to determine biofilm formation.

Results: Of the 49 isolates examined, 24 (48%) exhibited enhanced biofilm formation capacity relative to a standard *A. baumannii* strain (ATCC 19606). All isolates were carbapenem resistant *A. baumanii* and 38 (77%) were collected from patient in intensive care unit and a total of 47 (95%) patients exposed antibiotics within 1 month. The median duration of colonization was longer in biofilm producing isolates (18 vs. 12 days, p=0.044). Simultaneous colonization with other bacteria was more common in biofilm producing isolates (75% vs. 44%, p=0.042). The most prevalent co-colonization bacteria was *Stapylococcus aureus* (67%). The median day from admission to acquisition is seemed to be short in biofilm producing isolates which was not statistically significant (9 vs. 12days).
Conclusion: The biofilm production made the *A. baumannii* colonized more longer duration. During the colonization, also persuade co-colonization with other bacteria, especially *S. aureus*. Additional research is needed on possible links between biofilm formation and nosocomial infection.

P1-GR50

Current status of carbapenem-resistant *Enterobacteriaceae* (CRE) in the United States – analysis of TEST data

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Background: Carbapenem resistance has emerged and is disseminating among *Enterobacteriaceae*. Tigecycline Evaluation Surveillance Trial (TEST) data were used to evaluate the current status of resistance among CRE in the US since 2004.
Methods: MIC values were determined locally at 219 sites for 10,804 isolates using broth microdilution and following CLSI guidelines. 166 CRE were analyzed from a collection of 10804*Enterobacteriaceae* from 219 cumulative sites in the USA 2011-2014. Carbapenem resistance was determined by meropenem susceptibility. MIC values were determined by broth microdilution and interpreted using current CLSI guidelines.
Results: 166 of the 10,804 (1.5%) isolates were CRE, and of these 119 CRE (72%) were *K. pneumoniae*. The %S for various agents are shown in Table 1.

Table 1.

Organism/ESBL (N)	TIG	AK	CPM	CTR	LVX	MIN	P/T
Enterobacteriaceae (166)	**90.6**	85.6	12.7	2.2	16.6	63.5	7.2
CRE/ESBL Isolates (50)	**96.0**	82.0	2.0	0.0	2.0	58.0	0.0
Enterobacter spp.(27)	81.5	**92.6**	37.0	0.0	37.0	29.6	11.1
E. coli (15)	80.0	80.0	13.3	6.7	20.0	80.0	13.3
CRE/ESBL (2)	**100**	50.0	0.0	0.0	0.0	**100**	0.0
CRE/non ESBL (13)	76.9	84.6	15.4	7.7	23.1	76.9	15.4
K. pneumoniae (119)	**96.6**	84.0	3.4	0.0	4.2	67.2	0.8
CRE/ESBL (47)	**95.7**	83.0	2.1	0.0	2.1	57.5	0.0
CRE/non ESBL (72)	**97.2**	84.7	4.2	0.0	5.6	73.6	1.4

TIG, tigecycline; AK, amikacin; CPM, cefepime; CTR, ceftriaxone; LVX, levofloxacin; MIN, minocycline; P/T, piperacillin-tazobactam; ESBL, extended spectrum beta-lactamase positive. Bold = %S ≥90.

Conclusions: The CRE phenotype was relatively uncommon, but when encountered was predominately found among *K. pneumoniae*. Against CRE TIG was clearly the most potent agent, based on %S. Given the therapeutic challenges presented by CRE continued monitoring of their prevalence and potential spread is warranted.

P1-GR51

Antimicrobial susceptibility status of multidrug resistant (MDR) *Enterobacteriaceae* collected in Europe and North America: analysis of TEST program data (2010-2014)

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Background: The increase and spread of multidrug resistant (MDR) *Enterobacteriaceae* is a major concern worldwide. These pathogens are responsible for a variety of serious infections and based upon resistance to several antimicrobial classes are difficult to manage, often resulting in poor patient outcome. Data from the Tigecycline Evaluation Surveillance Trial (TEST) program were analyzed to evaluate the current status and antimicrobial susceptibility of MDR *Enterobacteriaceae* from Europe (EU), and North America (NA).
Methods: From 2010 to 2014, 50,435 isolates of *Enterobacteriaceae* from numerous countries in EU and states or provinces in NA were locally collected, identified, and susceptibility tested using broth microdilution and CLSI guidelines. MDR was defined as resistance to drugs from three or more different antimicrobial classes. The data were analyzed centrally at IHMA to determine the overall MDR rates and the activities of individual agents.
Results: Of the 50,435 *Enterobacteriaceae* isolates, 35,097 were collected in EU and 9,892 (28.2%) had a MDR phenotype. 15,338 were collected in NA and 2,731 (17.8%) had a MDR phenotype. Antimicrobial susceptibility profiles for all *Enterobacteriaceae* and the MDR population are shown in Table 1.

Table 1.

	All *Enterobacteriaceae*				MDR *Enterobacteriaceae*			
	EUROPE (35,097)		NA (15,338)		EUROPE (9,892)		NA (2,731)	
Compound	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline[a]	97.4	1	97.3	1	93.0	2	91.5	2
Pip-Tazo	83.5	64	90.6	16	47.8	> 128	53.5	> 128
Amikacin	98.2	4	99.3	4	94.0	16	96.4	16
Cefepime	87.0	32	94.4	2	59.8	> 32	71.8	> 32
Ceftriaxone	70.6	> 32	82.3	32	12.1	> 32	15.6	> 32
Levofloxacin	79.3	> 8	84.5	8	43.2	> 8	50.1	> 8
Meropenem	97.3	0.12	98.2	0.12	90.5	1	90.3	1

[a]FDA breakpoints used for tigecycline

Conclusions: The MDR rate among *Enterobacteriaceae* was higher in EU in comparison to NA. Amikacin, meropenem and tigecycline were the most active drugs *in vitro* against the MDR population in both regions. The prevalence and importance of MDR *Enterobacteriaceae* warrants ongoing surveillance and determination of the responsible resistance mechanisms.

P1-GR52

Antimicrobial susceptibility status of multidrug resistant (MDR) *Enterobacteriaceae* collected in Latin America: five year analysis of TEST program data (2010-2014)

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Background: Multidrug Resistant (MDR) *Enterobacteriaceae* have become a global problem. Multiple resistance mechanisms are often carried on plasmids and transposons that can provide isolates with resistance to several clinically important drug classes. These mobile genetic elements can rapidly disseminate into other species and can become a country specific or a regionally endemic

problem. Data from the Tigecycline Evaluation Surveillance Trial (TEST) program were analyzed to evaluate the contemporary prevalence and antimicrobial susceptibility of MDR pathogens from countries in Latin America.

Methods: Between 2010 and 2014, 3,439 isolates of *Enterobacteriaceae* from Argentina, Brazil, Chile, Colombia, El Salvador, Guatemala, Mexico, Panama, and Venezuela were locally collected, identified, and susceptibility tested using broth microdilution according to the CLSI guidelines. The data were centralized at IHMA for analysis of the MDR pathogens in each region. MDR was defined as resistance to drugs from three or more different antimicrobial classes.

Results: Of the 3,439 *Enterobacteriaceae* isolates 1,563 (45.4%) had a MDR phenotype (Table 1).

Table 1.

	All *Enterobacteriaceae* (3,439)			MDR *Enterobacteriaceae* (1,563)		
Drug	%S	MIC_{50}	MIC_{90}	%S	MIC_{50}	MIC_{90}
Tigecycline[a]	96.5	0.5	2	93.0	0.5	2
Pip-Tazo	76.7	4	> 128	51.9	16	> 128
Amikacin	93.3	2	16	86.4	4	32
Cefepime	77.9	≤ 0.5	> 32	54.2	8	> 32
Ceftriaxone	55.3	0.5	> 32	13.3	> 32	> 64
Levofloxacin	63.6	0.5	> 8	31.8	> 8	> 8
Meropenem	96.1	≤ 0.06	0.25	91.5	≤ 0.06	1

[a]FDA breakpoints used for tigecycline

The highest percentage of MDR *Enterobacteriaceae* was observed in Guatemala 67%, Panama 54%, and Chile 46.6%. Based on %S, amikacin, meropenem and tigecycline were the most active drugs *in vitro* against the MDR pathogens in this region.

Conclusions: The MDR rate among *Enterobacteriaceae* was notably high in Latin America, on average approaching 50%.The mobility of populations between regions and the critical importance of MDR *Enterobacteriaceae* on public health warrants continued monitoring in an ongoing manner.

P1-GR53

In vitro activity of tigecycline and comparators against *K. pneumoniae* and *K. oxytoca* collected from European countries: TEST 2010-2014

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Objectives: *K. pneumoniae* and *K. oxytoca* cause severe infections in hospitalized patients including a spectrum of clinical complications such as pneumonia, bacteremia, urinary tract and wound infections. In this study, data from the Tigecycline Evaluation Surveillance Trial (TEST) program were analyzed to evaluate the susceptibility profiles of *K. pneumoniae* and *K. oxytoca* isolates from European countries during the past five years.

Methods: From 2010 to 2014, isolates of *K. pneumoniae* (7,421) and *K. oxytoca* (2,361) were collected and susceptibility testing against tigecycline and comparators. Testing was performed locally using CLSI broth microdilution methods. CLSI and EUCAST breakpoint criteria were applied for comparison purposes.

Results: The activities of several antibiotics against *K. pneumoniae* and *K. oxytoca* are shown in Table 1.

Conclusions: *Klebsiella* spp. are common pathogens that can express resistance to a broad spectrum of antimicrobials. Against both *K. pneumoniae* and *K. oxytoca* the most active drugs (based on %S) were tigecycline, amikacin, and meropenem. Even though the %S rates varied with the use of CLSI/FDA interpretive breakpoints versus EUCAST breakpoints, the same three drugs (tigecycline, amikacin, meropenem) remained the most active. Because of the propensity of Klebsiella spp. to develop resistance the patterns observed to date could change, thus continued monitoring through surveillance is warranted.

Table 1.

	K. pneumoniae (7,421)					*K. oxytoca* (2,361)				
	CLSI		EUCAST			CLSI		EUCAST		
Compound	%S	%R	%S	%R	MIC_{90}	%S	%R	%S	%R	MIC_{90}
Tigecycline	95.2[a]	0.6[a]	85.2	4.8	2	99.1[a]	0.1[a]	95.6	0.9	1
Pip-Tazo	75.7	18.3	70.7	24.3	>128	82.9	14.6	81.6	17.1	>128
Amikacin	95.4	1.8	90.1	4.7	8	99.7	0.2	99.2	0.3	4
Cefepime	72.5	24.0	65.0	30.8	>32	96.6	2.0	87.3	5.4	2
Ceftriaxone	63.8	35.5	63.8	35.5	>32	81.7	15.9	81.7	15.9	16
Levofloxacin	71.2	24.7	68.7	28.8	>8	93.4	4.8	90.9	6.6	1
Meropenem	91.3	7.8	92.2	6.8	0.5	99.2	0.6	99.5	0.2	0.12

[a]FDA breakpoints used for tigecycline

P1-GR54

Bench and save carbapenems!

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Background: In the era of increasing carbapenem resistance, exploring alternative options is the need of the hour. Beta lactam-betalactamase inhibitors have retained sensitivity over time, against most Gram-negative bacteria.

Methods: This is a retrospective study done in a tertiary care oncology centre in India. Susceptibility pattern of *E.Coli, Klebsiella, Acinetobacter and Pseudomonas* blood culture isolates, identified between Jan 2013 to Jun 2014 were analysed using VITEK 2 compact autoanalyser. Colistin sensitivity was done using E test. Breakpoints for cefoperazone–sulbactam(CS) and cefepime/tazobactam(CT) are not elucidated in CLSI guideline and so breakpoints of cefoperazone and cefepime were applied.

Results: A total of 231 gram negative bacteremic isolates were analysed; *E.coli*81(ESBL 67),*Klebsiella*78 (ESBL 58), Pseudomonas 44and *Acinetobacter* 28. CT and carbapenam sensitivities were similar among ESBL *E.coli* isolates, with good sensitivity to aminoglycosides and chloramphenicol. ESBL *Klebsiella* also had similar susceptibility to carbapenem and CT. *Pseudomonas* showed 90% susceptibility to PTZ(piperacillin-tazobactam). Susceptibility to CT(88.6%) was better than imipenem(77.2%).*Acinetobacter* retained a higher sensitivity to CT(39.2%) than imipenem(32%).

Conclusion: The data reveals similar susceptibility of BL-BLI agents and carbapenem in *Enterobacteriaceae* and better susceptibility in the case of non fermenters(especially CT). This compelling laboratory data on the superior susceptibility makes a strong argument for using BL-BLI agents, especially CT and sparing carbapenem to curtail the spiraling carbapenem resistance.

P1-GR55

Molecular and biochemical characterization of a novel carbapenemase from *Acinetobacter baumannii* clinical isolates

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Carbapenem-hydrolyzing class D β-lactamases (CHDLs) have been reported increasingly and common among *Acinetobacter baumannii* isolates. Here, we report a novel CHDL identified in a carbapenem-resistant*A. baumannii* clinical isolate from Korea.

A total of 38 non-duplicate and carbapenem-resistant *A. baumannii* were recovered from a University hospital in Korea. MICs of the

antimicrobial agents were determined, according to guidelines of CLSI, by an agar dilution method. Molecular characterizations of β-lactamases were performed by PCR amplification, DNA sequencing, and Southern blot analysis. The $bla_{OXA-418}$ gene was expressed by a pET-30 system and the gene product was purified by His-Bind column and Mono S column. Steady-state kinetic constants of the purified enzyme were determined by fitting the initial rates directly to the Henri-Michaelis-Menten equation using nonlinear regression with the program DYNAFIT.
Among 38 isolates, one carbapenem-resistant *A. baumannii* harbored a novel variant ($bla_{OXA-418}$) of OXAs, which was encoded by the chromosome. The clinical isolate and its transformant showed resistance to carbapenems (especially to meropenem). Notable changes in MIC values were in line with the respective kinetic parameter differences. OXA-418 was most closely to OXA-228.
OXA-418 was derived from OXA-228 by the five substitutions (Val25Glu, Ser192Arg, Asp201Asn, Glu227Lys, and Asn257Asp; OXA numbering system). Among these substitutions, Asp201Asn and Glu227Lys are new sites for carbapenem resistance among OXA-228-like genes.

P1-GR56

Risk factors of MDR Gram negative bacteremia among hospitalized patients

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Background: Over the past decade, the numbers of bloodstream infections caused by multidrug-resistant (MDR) Gram-negative bacteria have risen sharply. MDR Gram-negative bacteremia increases not only mortality, but also patient morbidity, length of treatment and hospitalization costs. It is important to identify risk factors of MDR Gram-negative bacteremia among hospitalized patients to prevent these risk factors and to lower the incidence of MDR Gram-negative bacteremia.
Aim: To identify the risk factors of MDR Gram-negative bacteremia among hospitalized patients
Method: Risk factors were identified by a case-control study. Data was collected from inpatients medical record that had positive blood cultures of Gram negative bacteria from 2008-2013. The case group was subjects who had MDR Gram-negative bacteremia, and the control group was subjects who had non-MDR Gram negative bacteremia. All variables that had a value of p <0.25 on bivariate analysis were included in multivariate analysis using logistic regression.
Result: During the study period, there were 131 patients fulfilled the criteria: 42 patients who had MDR Gram-negative pathogen bacteremia (case) and 89 patients who had non-MDR Gram-negative pathogen bacteremia (control group). Based on the bivariate analysis, two variables were statistically significance: history of treatment in ICU/HCU (p=0.003) and history of ventilator (p=0.030). Further multivariate analysis showed that there was one variable statistically significance, which was history of treatment in ICU / HCU (OR: 3.118; CI 95% : 1.443–6.736; p=0,004).
Conclusion: History of treatment in ICU / HCU was risk factor of MDR Gram negative bacteremia among hospitalized patients.
Keywords: bacteremia, Gram negative, MDR, risk factor

P1-GR57

Analysis of the roles of small noncoding RNAs in responses to antibiotics and their applications on erradicating MDR bacteria

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The misuse of antibiotics has resulted in increasing bacterial multidrug resistance (MDR). Small noncoding RNAs (sRNAs), modulators of multiple cellular events, may influence bacterial responses to antibiotics; however, their roles and mechanisms of action remain largely unknown. Here, the susceptibilities of *Escherichiacoli* strains overexpressing each of the 26 known Hfq-dependent sRNAs to major classes of antibiotics were determined. The results suggested that 17 sRNAs modulate antibiotic susceptibility; overexpression of nine of these sRNAs specifically reduced or potentiated antibiotic efficacy. These phenotypes were conserved between species, but the essentialities of the sRNAs were limited. Based on overexpression and knockout studies, the results presented here, firstly, unveil sRNA-mediated modulatory pathways and, secondly, suggest that the sRNAs could be used as biomarkers to identify cephalothin-resistant strains. Furthermore, the sRNAs may potentiate the effect of levofloxacin, allowing the modulation of antibiotic action on MDR strains. In summary, sRNAs have the potential to enable bacteria to adapt smartly to antibiotic challenges via multifaceted approaches.

P1-GR58

Clinical and microbiological characteristics of *Klebsiella pneumonia* bacteremia in a single center

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Background: The aim of present study is to evaluate clinical and microbiological characteristics of *K. pneumonia* bacteremia in a single center.
Methods: The study was conducted at Keimyung University. All episodes of *K. pneumonia* bacteremia (n = 344) during two 2 year periods (2004-5 and 2012-3) were retrospectively compared.
Results: Of a total of 344 patients, 140 (40.6%) were the first period and 204 (59.4%) were the second period. The frequencies of cardiovascular diseases (47.5% vs. 24.3 *P*=0.001), neurologic diseases (19.8% vs. 10.8% *P*=0.035) were significantly higher in the second period than in the first period. The frequencies of cefotaxime (20.1% vs. 7.1% *P*=0.001), cefepime resistance (19.1 vs. 0% *P*=0.001) and ESBL positivity (19.1% vs. 4.3% *P*=0.001) were significantly higher in the second period than in the first period. But the initial empirical antibiotic use of cefepime (17.9% vs. 0% *P*=0.001), carbapenem (19.8% vs. 2.9% *P*=0.001), vancomycin (12.4% vs. 4.3% *P*=0.012) were higher in the second period than in the first period. In the subgroup analysis, the frequencies of cefotaxime (32.4% vs. 10.6% *P*=0.002), cefepime resistance (30.6% vs. 0% *P*=0.001) and ESBL positivity (31.5% vs. 6.1% *P*=0.001) were higher in the second period than in the first period in the healthcare associated *K. pneumonia* bacteremia group. But the initial empirical antibiotic use of cefepime (17.9% vs. 0% *P*=0.001), carbapenem (19.8% vs. 2.9% *P*=0.001) were higher in the second period than in the first period in both groups.
Conclusion: The frequencies of antibiotic resistance in the community associated *K. pneumonia* bacteremia group did not differ between the first period and the second period. But the initial empirical antibiotic use of cefepime (17.9% vs. 0% *P*=0.001), carbapenem (19.8% vs. 2.9% *P*=0.001) were higher in the second period than in the first period in both groups.

P1-OB01

High bacterial resistance in surgical site infections in Benin

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Background: Despite advances in surgical technique, antibiotic and recent therapeutic measures, infection is still a major problem in surgery. In developing countries, Surgical Site Infections (SSI) are the most common healthcare associated infections and an obsession for the surgical staff. This study aims to describe the bacteriological aspects of SSI at the county hospital of Borgou in Benin.

Methods: A prospective and descriptive study about 44 cases of SSI from February to August 2013.

Results: The frequency of surgical site infections was 7.3% of operated (44/603). The superficial incisional infections were 6, 34 deep and organ or space infections were 4. Culture was sterile in 12 cases (27.3%) and positive in 32 cases (72.7%). In two cases were isolated two germs simultaneously. Gram negative organisms were more isolated with 22 cases (64.7%) of *Escherichia coli*. Multidrug resistance was observed in 14 of 34 cases (41.2%).

Conclusion: Gram negative were the predominant bacteria in surgical site infections at county hospital Borgou. The high rates of resistance to common antibiotics must arouse Ardies preventive action to influence the frequency curve and protect antibiotics.

P1-OB02

Epidemiology of etiology and resistance patterns of bacteremia in children with hematology-oncology disease: a retrospective single center study

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Objective: Knowing the epidemiology of infection and antimicrobial resistance pattern in individual institute is important to guide optimal empiric antimicrobial choice, particularly in high risk patients. In addition, this epidemiological pattern continues to change over the years. We assessed changes in etiology of bacteremia and the resistance patterns in pediatric cancer patients.

Methods: Pediatric hematology-oncology patients with bacteremia were included at Samsung Medical Center. We investigated the bacteremia episodes that occurred in 2004 and 2013.

Results: During the study period, a total of 201 bacteremia episodes were documented in 144 patients; a total of 55 patients had 66 bacteremia episodes in 2004; 89 patients had 135 bacteremia episodes in 2013.

The median age at bacteremia was 8.4 years and male was 67.7% (136/201). Gram-positive organisms isolated in 52.2% (105/201) of bacteremia. The incidence of methicillin-resistance *staphylococcus aureus* (MRSA) was 50% (1/2) of *S. aureus* bacteremia in 2004 and 38.8% (7/18) in 2013. In case of gram-negative organisms, cefepime-resistance rates in gram-negative organism were 3.8% (1/26) in 2004 and 18.2% (12/66) in 2013 (P=0.10). Piperacillin/tazobactam resistance rates were 3.8% (1/26) in 2004 and 10.6% (7/66) in 2013(P=0.43).

Conclusion: Although not significant, trends were observed for increasing resistance rate in cefepime and piperacillin/tazobactam among gram-negative bacteria isolated from the pediatric cancer patients in our center. Because these two antibiotics are main agents for initial choice for neutropenic fever in our center, a continuous monitoring is needed to reveal any significant increase for resistance.

P1-OB03

Implication of use of glutaraldehyde based high-level disinfectants at high temperatures for combating microbial resistance

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High-level disinfectants (HLD) used to disinfect semi-critical medical devices must demonstrate the ability to inactivate mycobacteria. Manufacturers of HLDs must specify use conditions such as temperature, concentration and contact time by demonstrating elimination of acid-fast mycobacteria, which are indicators of resistance to disinfection. An inverse relationship between temperature and disinfection time was shown with a typical glutaraldehyde formulation in a quantitative suspension test against *Mycobacterium terrae*. Other seemingly resistant strains of mycobacteria demonstrate similar behavior, indicating that marginal heating above room temperature plays a crucial role in effectiveness. However, heating (~20°C above ambient) of HLDs could increase the amount of vapors in poorly ventilated areas for all disinfectants irrespective of its active ingredient. Although a long and successful use history has been documented for glutaraldehyde formulations, a US FDA user experience database shows an increase in respiratory and dermal health effects for personnel who disinfect endoscopes without adequate controls. Lack of proper ventilation, appropriate personal protective equipment (PPE), and inappropriate responses to glutaraldehyde spills have been identified as significant root causes. A control plan, in conjunction with good ventilation and proper PPE use, can help prevent needless occupational asthma and dermal sensitization. To aid in this effort, a "Glutaraldehyde Spill Calculation Tool" was developed and validated in an empty endoscope reprocessing room. Parameters such as air-exchange rates, size of spill, time of clean-up and concentration of disinfectant were varied while collecting air samples. Use of this model could successfully predict expected levels of glutaraldehyde concentrations in the workspace before adverse health affects occur.

P1-OB04

The serogroup and antibiotic resistance of *salmonella* species isolated in a single center

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Background: The incidence of typhoid fever is decreased in Korea. However, nontyphoidal salmonellosis occurs persistently. Therefore, we investigated the serogroup and antibiotic resistance pattern of salmonella species isolated from pediatric patients in our center.

Methods: We studied the salmonella isolates from stool, blood, and other sites of pediatric patients who were evaluated at Samsung Medical Center, Seoul, Korea from January 2001 to December 2014. In addition to routine culture procedures for blood and other sites, stool specimens were inoculated to MacConkey agar, Salmonella-Shigella (SS) agar, and Selenite F (SF) broth. We used Vitek II-GN to isolate salmonella and further analyzed the serogroup of salmonella isolates. Antibiotic resistance was tested by Vitek GNS card.

Result: A total of 259 salmonella isolates were identified from 238 pediatric patients from January 2001 to December 2014.

Nontyphoidal salmonellosis was diagnosed in 232 patients (97.5%) and typhoid fever due to *S.typhi* infection was identified in 6 patients (2.5%). Among nontyphoidal salmonella species, serogroup D occupied the highest proportion (42.9%). The proportions of serogroup C and B were 23.2%, and 20.1%, respectively. The antibiotic resistance rates of nontyphoidal salmonella species were 44.6% to ampicillin, 4.8% to trimethoprim/sulfamethoxazole, 1.2% to ciprofloxacin, and 6% to ceftriaxone, respectively. The ampicillin resistance rates according to serogroup were 55.8% in serogroup B, 23.8% in serogroup C, and 56.8% in serogroup D. The trimethoprim/sulfamethoxazole resistance rates were 11.5% in serogroup B, 7.7% in serogroup E, and 3.6% in serogroup D. Among eight *S.typhi* isolates, one isolate had resistance only to ampicillin and the rest of the seven isolates were all sensitive to ampicillin, trimethoprim/sulfamethoxazole, ciprofloxacin, and ceftriaxone.
Conclusions: Nontyphoidal salmonella species was identified as the main pathogen in pediatric salmonella infection of our center (232/238, 97.5%). And serogroup D was most commonly isolated (111/259, 42.9%). The antibiotic resistance rates of nontyphoidal salmonella species was the highest to ampicillin (44.6%). A continuous monitoring of antibiotic resistance among salmonella species is needed.

P1-OB05
Molecular detection of *Clostridium difficile* ribotype NAP-1/027 strain in University Malaya medical centre
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Antibiotic-associated diarrhea and colitis were established soon after the widespread use of antibiotics.NAP-1 strain characterized by 18-bp deletion at position 330 to 347 of *tcdC* gene and resistant to moxifloxacin is associated with hyperproduction of toxin and disease severity. This study aimed to detect the presence of the NAP-1 strain among stocked isolates of *C. difficile* from University Malaya Medical Centre (UMMC). Thirty *C. difficile* isolates were obtained from the stock culture collection from 2007 to 2010. Toxin A, B and *tcdC* genes were amplified using polymerase chain reaction (PCR). Presence of any *tcdC* gene deletion was identified by sequencing. Moxifloxacin resistance was performed using E-test to determine the minimum inhibitory concentrations (MIC). Twenty isolates were found to carry the toxin A, toxin B, and *tcdC* genes. The *tcdC* genes, which codes for the negative regulators, were also found in the same isolates that had toxin A and B genes. Analysis of sequencing of the *tcdC* gene for the toxigenic isolates did not reveal any 18-bp deletion at position 330 to 347. Four isolates were resistant to moxifloxacin with MIC ≥8 μg/ml. The NAP-1 strain was not detected among the isolates however four isolates demonstrated moxifloxacin resistant. This finding suggests that UMMC does not have NAP-1strain, and moxifloxacin resistant most probably associated with other important PCR ribotypes circulating in the hospital settings.

P1-OB06
First survey of antibiotic susceptibility of anaerobic bacteria in a large tertiary hospital in Singapore
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Anaerobic bacteria may cause serious infections such as intra-abdominal infections and brain abscesses. Due to the fastidious nature of anaerobic bacteria, *in vitro* antibiotic susceptibility testing is costly and laborious and is not performed in most routine clinical laboratories. Therefore, the treatment for anaerobic infections is empirical, with antimicrobial agents thought to be predictably efficacious against anaerobes. However, with increasing antimicrobial resistance reported among anaerobes against commonly used empiric antibiotics, there is an urgent need to detect any emerging resistance in our institution. This study aims to survey the antimicrobial susceptibility profiles of clinically significant anaerobes isolated in Singapore General Hospital.
Antimicrobial susceptibility testing is performed on 120 clinical anaerobic isolates collected from July to December 2014. A panel of antibiotics including benzylpenicillin, amoxicillin-clavunalate, piperacillin-tazobactam, imipenem, metronidazole, clindamycin and moxifloxacin is tested using the Epsilometer (Etest) method. Minimum inhibitory concentration (MIC) endpoints are interpreted using the Clinical and Laboratory Standards Institute (CLSI) guideline. American Type Culture Collection strains of *Bacteroides fragilis* ATCC 25285 and *Eggerthella lenta* ATCC 43055 are included as quality controls. In addition, the presence of β-lactamase is tested in each isolate using the cefinase disc method.
The antimicrobial susceptibility profiles of the tested isolates will be presented. Resistance to multiple commonly used antimicrobial agents including imipenem and metronidazole is detected in *Bacteriodes species*.
Routine susceptibility testing may be required for critical anaerobic infections for optimal patient management.

P1-OB07
Antibiotic susceptibility and potential probiotic properties of *Lactobacillus sakei* isolates originated from dry fermented sausages
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Biopreservation is an innovative method of extending shelf life of food products and reducing microbial risks. It refers to inoculation of food products with selected bacteria able to inhibit the growth of undesirable bacteria. However, bacteria are highly adaptable and are capable of developing resistance to antibiotics. Antibiotic resistance transmission from different kinds of food to the consumer is a matter of public health. The objective of the present study was the evaluation of probiotic properties and antibiotic susceptibility of two *Lb. sakei* isolates originating from dry fermented sausages produced without starter cultures. Isolates were tested for antibiotic resistance against the 18 antibiotics by the disk diffusion method. Growth in low pH values and bile salts presence were measured. One of the isolates showed potential probiotic properties, but it was resistant to amoxicillin, ampicillin, kanamycin, vancomycin, penicillin, ciprofloxacin, neomycin, nalidixic acid and sulfomethoxazole. The second isolate showed potential probiotic properties and resistance to less antibiotics than the other isolate and especially to the three critically important antimicrobials (macrolides, 3rd-4th generation cephalosporins and quinolones), so it is a possible candidate for further investigation.
Acknowledgement: Research project co-financed by the European Union (European Regional Development Fund – ERDF) and Greek national funds through the Operational Program "Competitiveness and Entrepreneurship" of the National Strategic Reference Framework (NSRF) 2007-2013 – National Action "Cooperation 2011: Partnerships of Production and Research Institutions in Focused Research and Technology Sectors of General Secretariat for Research and Technology.

P1-OB08

Antibiotic susceptibility of lactic acid bacteria isolated from fermented sausages produced without starter cultures

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Dry fermented sausages produced without starter cultures constitute useful sources for isolation of novel lactic acid bacteria (LAB) with probiotic properties. These bacteria originate from autochthonous cultures of meat or from the residual microflora of surfaces and equipment of production units, and are very promising for future use in sausage production with improved organoleptic, sensory and health promoting characteristics. To prevent the undesirable transfer of resistance to endogenous bacteria, lactic acid bacteria should not carry resistance other than that required. The objective of the present study was the evaluation of antibiotic susceptibility of lactic acid bacteria with potential probiotic properties, originating from fermented sausages. A total of 7 isolates able to grow in low pH values and bile salts presence were chosen and subjected to disc diffusion tests in order to evaluate their antibiotic susceptibility to several antibiotics. All isolates were found susceptible to streptomycin, gentamycin, tetracycline, chloramphenicol, cephalothin, clindamycin, erythromycin and cefotaxime and resistant to ampicillin, kanamycin, vancomycin and sulfomethoxazole. *Lb. casei* (n = 1), *Lb. sakei* (n = 1) and *Ped. pentosaceus* (n = 1) showed resistance to less antibiotics than the rest of the isolates examined and especially to the three critically important antimicrobials (macrolides, 3rd-4th generation cephalosporins and quinolones).

Acknowledgement: Research project co-financed by the European Union (European Regional Development Fund – ERDF) and Greek national funds through the Operational Program "Competitiveness and Entrepreneurship" of the National Strategic Reference Framework (NSRF) 2007-2013 – National Action "Cooperation 2011: Partnerships of Production and Research Institutions in Focused Research and Technology Sectors of General Secretariat for Research and Technology.

P1-OB09

Antibiotic susceptibility of a probiotic and other lactobacilli isolated from fermented sausages

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Background: The food chain has been recognized as one of the main routes for the transmission of antibiotic-resistant bacteria. Starter –culture bacteria deliberately introduced in the food chain might pose a risk to human and animal health through the carrying of resistance genes. Fermented meats that are not heat-treated provide a vehicle for such bacteria and can act as a direct link between the indigenous microbiota of animals and the human GI tract. Lactobacilli are generally recognized as safe and are not responsible for human infections. However, under certain conditions they can act as reservoir of transmissible antibiotic resistance genes.

Methods: In this vain, strains of lactobacilli among which a probiotic strain of *Lactobacillus paracasei* K5, were isolated from 15 samples of fermented sausages and tested for their antibiotic resistance against a battery of classical antibiotics. Our study was focused on the possible resistance exhibited by the probiotic strain used as a starting culture and into the final product in an attempt to reveal any alterations occurring during the fermentation period. Lactobacillus spp were initially isolated in MRS, identified with biochemical profiling and molecular methods (for K5 strain) and the susceptibly to antibiotics was estimated by the E-test approach.

Results/Conclusions: According to our results, *L. paracasei* K5 was susceptible to all antibiotics tested ensuring the safety of the product. However various results were obtained concerning other Lactobacillus spp.

Acknowledgement: Research project co-financed by the European Union (European Regional Development Fund – ERDF) and Greek national funds through the Operational Program "Competitiveness and Entrepreneurship"

P1-OB10

Antimicrobial resistance and detection of mutation of non typhoidal *salmonella* isolates: unrelenting challenge

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Antimicrobial chemotherapy can be lifesaving in bacteraemia caused by non-typhoidal *Salmonella* (NTS). However, the efficacy of chemotherapy can be compromised by drug resistance. This study was undertaken to describe the resistance profiles and resistance mechanism of clinical NTS isolates. Thirty isolates of NTS were isolated from blood (19), stool (10) and bronchi alveolar lavage (BAL; 1) between December 2011 and December 2012. These isolates were tested for susceptibility to ampicillin, gentamicin, tetracycline, co-trimoxazole, nalidixic acid, ciprofloxacin and ceftriaxone by disc diffusion method. E-test for nalidixic acid and ciprofloxacin were performed for nalidixic acid resistant isolates. Mutations within quinolones- resistance determining regions were performed by sequencing *gyrA*, *gyrB*, *parC* and *parE* genes. Resistance rates of NTS isolates from blood, stool, and BAL were respectively 37%, 20% and 0% for ampicillin, 79%, 40% and 0% for tetracycline, 32%, 40% and 0% for co-trimoxazole, 37%, 10% and 100% for nalidixic acid. All the isolates were susceptible to gentamicin, ciprofloxacin and ceftriaxone. Six isolates were resistant to nalidixic acid, whereas two isolates were susceptible by E-test. Seven isolates exhibited reduced susceptibility towards ciprofloxacin, while the remaining was susceptible. A single mutation in *gyrA* gene was detected in six isolates that involved four amino acid substitutions, while in *parE* gene three isolates had a single base insertion. However there was no mutation in the other two genes.Mutation in *gyrA* was sufficient to induce decreased susceptibility to ciprofloxacin. Other gene mutation and mechanism are responsible for this phenomenon, as evident by mutation in *parE* gene.

P1-OB11

Activity of tigecycline and comparators against Gram-negative anaerobic pathogens from 2013-2014

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Background: Anaerobic pathogens are commonly involved in polymicrobial infections. Tigecycline (TCG) is a broad spectrum agent targeting both gram-positive and -negative aerobic and anaerobic bacterial species. This report evaluates the *in vitro* activity of tigecycline and comparators against gram-negative anaerobic clinical isolates from skin/soft tissue (SSTI) and intra-abdominal (IAI) specimens from 2013-2014.

Methods: 609 gram-negative anaerobic isolates from unique patient infections in seven European countries (Belgium,

Czech Republic, France, Germany, Hungary, Spain and Sweden) were tested at a central laboratory by agar dilution following CLSI M11-A8 guidelines. Interpretation of susceptibility was determined using CLSI or FDA (TCG) breakpoint criteria.

Results:

Source (n)	Drug	%S	%I	%R	MIC_{50}	MIC_{90}
SSTI (281)	Cefoxitin	90.4	7.8	1.8	4	16
	Clindamycin	66.2	5.7	28.1	1	>8
	Meropenem	98.9	1.1	0	0.12	0.5
	Metronidazole	100	0	0	0.5	1
	Pip Tazo	98.9	1.1	0	0.25	8
	Tigecycline	99.3	0.7	0	0.25	2
IAI (328)	Cefoxitin	85.1	12.2	2.7	8	32
	Clindamycin	60.4	4.9	34.8	1	>8
	Meropenem	99.4	0	0.6	0.12	0.5
	Metronidazole	100	0	0	0.5	1
	Pip Tazo	97.0	3.1	0	0.5	16
	Tigecycline	96.7	3.4	0	0.25	2

Conclusions: The *in vitro* activity of agents tested against this contemporary collection of anaerobes was consistently high for most agents. Meropenem, metronidazole, piperacillin tazobactam and tigecycline inhibited >96% of isolates at their respective susceptible breakpoints. Cefoxitin and clindamycin were less active. There were statistically significant differences in the activity of cefoxitin and tigecycline between SSTI and IAI isolates ($p<0.05$, Fisher's exact test). As many therapeutic decisions on treatment of anaerobic or polymicrobic infections are empiric, continual monitoring of anaerobic bacterial susceptibility is essential.

P1-OB12

Antibiogram of biofilm producing clinical isolates from indwelling medical devices of patients in intensive care units

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Background: Microbial biofilms create great nuisance to microbiologists and clinicians as it is difficult to eradicate them. It is, therefore, crucial to know the drug resistance pattern of biofilm-producers so that the clinicians can choose appropriate antibiotics for the treatment of patient.

Methods: This prospective study included 100 samples showing bacterial growth from the intensive care units of two hospitals including a university hospital and a private hospital in Kathmandu. The isolates were identified following standard methodology and the antibiogram of the isolates were produced following Kirby-Bauer disk diffusion method. Detection of biofilms was done by tissue culture plate method.

Results: Out of 105 clinical isolates, 52 (49.5%) bacteria were biofilm producers. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Burkholderia cepacia* complex were found to be the most frequent biofilm producers. Higher antibiotic resistance was observed in biofilm producers than in biofilm non-producers. The most effective antibiotics for biofilm producing Gram-positive isolates were vancomycin, tigecycline and linezolid, and that for biofilm producing Gram-negative isolates were polymyxins and tigecycline. Among the multidrug resistant gram-negative bacteria, co-resistance was seen most commonly with amoxicillin, cotrimoxazole and ciprofloxacin.

Conclusion: Nearly fifty percent of the isolates were found to be biofilm producers. Meticulous decision must be taken for prescribing antimicrobials against biofilm-producing bacteria.

P1-OB13

Knowledge, belief and practice of interventions to contain antimicrobial resistance among physicians in Sokoto, northwestern Nigeria

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Keywords: Anti-microbial sensitivity test, antibiotics, healthcare workers, WHO global strategy

Background: Antimicrobial Resistance (AMR) continued to be a major public health problem worldwide.

Objectives: To assess the knowledge, belief and practice of WHO interventions to contain AMR among physicians in Sokoto State, Northwestern Nigeria.

Methodology: This is a cross-sectional study involving 105 physician's sampled for the current population of 400 physicians working across the state. A multi-stage sampling technique was used to select eligible participants for this study. Using self-administered questionnaire relevant data was obtained from the respondents. This study receives ethics approval from the Usmanu Danfodiyo University Teaching Hospital, Sokoto. Data analysis included mean, proportions, chi square test, independent sample t test and ANOVA.

Results: All the physicians were knowledgeable about AMR. We found 57.1% of the physicians lacks an up-to-date information on AMR. Majority of physicians (81.9%) had no training on AMR. Over 2/3rd (67.6%) use results of anti-microbial sensitivity test (AST) to guide patients anti-microbial treatment and 69.5% of the respondents were aware of general interventions to contain AMR. Similarly, 73.3% of the physicians were not aware of the WHO Global strategy for the containment of AMR. Majority of physicians agreed or strongly agreed AMR as worldwide and national problem but few considered AMR as problem in their own hospitals.

Conclusion: Majority of physicians lack knowledge on the WHO Global Strategy for the containment of AMR and up to-date knowledge on AMR. Self-prescription by patients and poor awareness on WHO global strategy for the containment of AMR are areas of interventions for prevention and control of AMR.

P1-SP01

Mupirocin resistance of methicillin-resistant *Staphylococcus aureus* in pediatric patients

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Background: Mupirocin is a topical antimicrobial agent which have been widely used to eradicate nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA). However, extensive use of mupirocin has been observed. Mupirocin abuse may facilitate emergence of mupirocin resistance in *S. aureus*. Therefore, we investigated the mupirocin resistance among MRSA isolates in pediatric patients.

Methods: A list of MRSA isolates was identified at the department of pediatrics, Samsung Medical Center from January 2011 to December 2013. MRSA isolates were tested for antibiotics as

per Clinical and Laboratory Standards Institute guidelines. A retrospective chart review was performed.
Results: A total of 896 MRSA isolates from 867patients were identified during the study period. The total number of patients who had mupirocin-resistant MRSA in any body site was 123 during study period. Median age of patients with mupirocin resistant MRSA and mupirocin sensitive MRSA was 0.17 years and 0.15 years, respectively. Among 123 patients, 91 patients were under 1 year old (91/123, 74%). Among the total MRSA isolates, 22 were blood isolates in 22 patients, and were all sensitive to mupirocin. Mupirocin resistance rate of MRSA was 14.3%, 11.0%, and 16.4% in 2011, 2012, and 2013, respectively. There was no difference for year-to-year mupirocin resistance rate distribution (P=0.174).
Conclusions: The rate of mupirocin-resistant MRSA of pediatric patients in a single tertiary center was 14.2% during the study time. Further surveillance of mupirocin-resistant MRSA is needed. Moreover, the more stringent guides for use of mupirocin should be established.

P1-SP02
The prevalence and antibiotic resistance pattern of MRSA (methicillin resistant *Staphylococcus aureus*) isolates from clinical specimens during 2010-2014 in General Hospital of Dr. Saiful Anwar Malang, East Java, Indonesia
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Methicillin Resistant *Staphylococcus aureus* (MRSA) has become the major problem worldwide as one of the most resistant infectious agent in antibiotics era. Our objective is to compare the antimicrobial resistance pattern of MRSA isolates in Dr. Saiful Anwar General Hospital, Malang, East Java, Indonesia during 2010-2014. The MRSA isolates were obtained from varies clinical specimens including blood, pus, sputum, and urine, which detected by cefoxitin disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI) 2011. Such antibiotic susceptibility test was performed toachieve the susceptibility profiles. We carried out the antibiotic susceptibility test over 295 MRSA isolates collected during 2010-2014, respectively. The MRSA prevalence tend to increase from year 2010 to 2012; 41.8%, 41.7%, and 45.3%, respectively. However the prevalence was decrease in 2013 (33.5%), but rise again in 2014 by 37%. The resistance rate towards chloramphenicol, erythromycin, tetracycline, and trimethoprim-sulfamethoxazole was significantly differing year by year in blood, pus, and sputum specimens ($p<0.05$). In conclusion, the antimicrobial resistance control program and surveillance in dr.Saiful Anwar General Hospital Malang, East Java, Indonesia, needs to be improved properly.

P1-SP03
The cefazolin inoculum effect in methicillin-susceptible *Staphylococcus aureus* blood isolates: their association with dysfunctional accessory gene regulator (*agr*)
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Purpose: We evaluated the clinical significance of the cefazolin inoculum effect (CIE) in MSSA isolates, with an emphasis on analyzing the relationship between the CIE and (accessory regulator gene) *agr* dysfunction.
Methods: In total, 146 isolates were recovered from patients with MSSA bacteremia in two multicenter surveillance studies at nine hospitals in Korea.
Results: The CIE was observed in 16 MSSA isolates (11.0%), and type A was the only detected β-lactamase in MSSA isolates exhibiting the CIE (100%), no strains expressing type B, C, or D β-lactamases exhibited this effect. Furthermore, the CIE was only observed in *agr* group III (12/16; 75.0%) and I (4/16; 25.0%) isolates, and was significantly more common in isolates with *agr* dysfunction than in those with functional *agr* (56.3% vs. 7.7%; $p < 0.001$). Even among isolates producing type A β-lactamase, the CIE was also prevalent in isolates with dysfunctional *agr* than in isolates with functional *agr* (69.2% vs. 14.9%, $p = 0.025$). Conversely, isolates expressing type B and D β-lactamases exhibited no *agr* dysfunction. While the CIE was not observed in spa type t126 isolates, 66.7% of the spa type t012 (4/6) and each of the spa type t021 (3/3) isolates showed the CIE.
Conclusions: This study demonstrates an association between the CIE of MSSA isolates and *agr* dysfunction, in addition to those between the CIE and type A β-lactamase. Moreover, the analysis of MSSA isolates highlights the predominance of particular strains that exhibit the CIE in Korea.

P1-SP04
Genetic analysis of *Staphylococcus aureus* strains isolated from patients admitted to Alzahra hospital in Isfahan
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Background: *Staphylococcus aureus* is a major human pathogen associated with a vast spectrum of community and hospital acquired infections. Therefore the study of its origin and resistance is of utmost importance for determining an appropriate treatment pattern. The aim of this study was the detection of methicillin resistance gene by PCR and its typing using SCC mec and Agr methods.
Method: A total of 100 clinical samples were collected from the patients admitted to various wards of Esfahan's Alzahra hospital. MIC to methicillin was determined using disc diffusion method. Thereafter the samples containing mecA gene were subjected to SCC mec and Agr typing. All samples were ultimately tested for antibiotic resistance using disc diffusion method.
Results: The frequency of mecA gene in *S. aureus* strains was found to be 28% using PCR (genotypic method) and resistance to oxacillin was determined 24% by agar dilution method (phenotypic method). Results by SCC mec typing revealed that the majority of strains were type IV and the minority were of type I whereas Agr typing showed type I to be predominant with the minority belonging to type III.
Conclusion: The resistance of *S. aureus* strains to antibiotics is on the increase and SCC mec type IV isolates, being CA_MRSA themselves, are continuing to spread in the communities. It should be noted that *S. aureus* strains in different regions have different agr patterns.
Keywords: *Staphylococcus aureus*, methicillin, mecA, SCC mec, Agr.

P1-SP05

Prevalence and characterization of *Staphylococcus aureus* causing skin and soft tissue infections in the community setting in Indonesia

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Staphylococcus aureus is an important pathogen associated with serious infections in the community. Little is known about the epidemiology of *S. aureus* in the community setting in Indonesia. This study aimed to define *S. aureus* obtained from community setting in Indonesia. Out patients presenting with skin and soft tissue infection (SSTI) in three cities were cultured for *S.aureus* in anterior nares, throat, and wound.257/567 (45%) *S. aureus* were isolated from wound, eight (3%) were methicillin-resistant (MRSA). The Panton-Valentine leukocidin (*pvl*) and exfoliative toxin genes were detected in 22% and 17% of methicillin-sensitive *S. aureus* (MSSA), respectively. Nasopharyngeal MSSA carriage was associated tothe *S.aureus* SSTI ($p<0.001$; $p<0.05$). Type of SSTI, previous antibiotic therapy and previous hospitalization were associated with *pvl* genes positive MSSA. Type III and IV SCC*mec* were found among MRSA strains.. Typing MRSA by Raman spectroscopy and multiple locus variable number tandem repeat analysis (MLVA) presented SIRU profile 6.4.5.3.3.11 for the five MRSA strains assigned to Raman type 24, indicating clonal spread. In conclusion, the prevalence of MRSA causing SSTI in the community was low but MRSA be spreading clonally. In contrast, the prevalence of PVL-positive MSSA among patients with *S.aureus* SSTI in community setting in Indonesia is high.

Keywords: Indonesia, *Staphylococcus aureus*

P1-SP06

The effect of daily chlorhexidine bathing on the acquisition of methicillin-resistant *Staphylococcus aureus* in the medical intensive care unit

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Background: Universal decolonization are recommended in intensive care unit (ICU) that have unacceptably high methicillin-resistant *Staphylococcus aureus* (MRSA) rates despite implementation of the basic prevention strategies.

Methods: An interrupted time-series study was performed to evaluate the effect of daily chlorhexidine bathing on the acquisition of MRSA in the medical ICU with MRSA endemicity. There was a 14-month control period and a 16-month chlorhexidine bathing period. Segmented Poisson regression analysis was performed to assess the impact of chlorhexidine bathing on the incidence density of MRSA. Also, chlorhexidine susceptibility testing was performed on MRSA isolates collected during chlorhexidine bathing period.

Results: There was a 23.6% reduction of MRSA acquisition rates after an introduction of daily chlorhexidine bathing (21.03 vs. 16.06 cases/1000 at-risk patient days, $p = 0.101$). There was a significant reduction in trend (-0.056; 95% CI -0.095 ~ -0.017; P=0.005) of incidence density of MRSA despite a significant increase in both level (0.358; 95% CI 0.063 ~ 0.654; P<0.017) and in trend (0.040; 95% CI 0.006 ~ 0.073; P<0.020) of MRSA prevalence rates during chlorhexidine bathing period. Minimum inhibitory concentration of chlorhexidine against a total of 204 MRSA isolates ranged from 2 to 4ug/mL. Minimal bactericidal concentration (MBC) of chlorhexidine ranged from 2 to 128 ug/mL, except 26 MRSA isolates with MBC of chlorhexidine > 128 ug/mL.

Conclusion: The acquisition rates of MRSA showed significantly decreasing trend in the medical ICU during chlorhexidine bathing period.

P1-SP07

Study on the MIC of vancomycin to *Staphylococcus aureus* isolated from the inpatients of Nguyen Tri Phuong hospital in HCMC – Vietnam

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Background: The current challenge is the increasing of antibiotic resistance among the bacterial pathogens. In particular, for *S. aureus* is the increase rate of MRSA and the decrease sensitivity of vancomycin, the first choice antibiotic for treatment of serious MRSA infections.

Objective: Update the situation of the antibiotic-resistant among *S. aureus,* especially susceptibility level to vancomycin

Material and methods: *S. aureus* isolated from inpatient at Nguyen Tri Phuong Hospital from 01/2012 to 01/2013. The identification of *S. aureus* were done by biochemical tests, the antimicrobial susceptibility testing were carried out by t*he Kirby – Bauer disk* - diffusion technique, and the MIC vancomycin were determined by the vancomycin E-test.

Results: A total of 147 *S. aureus* isolates from different specimens were collected. MRSA is 51%. The studied *S. aureus* were highly resistant to penicillin (#100%). The MRSA were highly resistant to erythromycin (92%), clindamycin (84%), ciprofloxacin (65.3%) and gentamicin (62.7%) compare to the MSSA: erythromycin (45.8%), clindamycin (61.9%), ciprofloxacin (44.9%) and gentamicin (40.8%). Most of the isolates were sensitive to vancomycin, rifampicin, and linezolide. MIC_{90} of vancomycin on *S. aureus* is 1 μg/mL. With MIC ≤1 μg/mL, there is no difference of vancomycin' MIC between MRSA and MSSA; but with vancomycin MIC higher than 1 μg/mL, MRSA seems to have the higher vancomycin's MIC. There were no VISA and VRSA reported in the study. The MRSA with vancomycin's MIC of 1-2μg/mL is 20%.

Conclusion: *S. aureus* including MRSA and are still sensitive to vancomycin. But MRSA with vancomycin's MIC of 1-2 μg/mL is 20% and there were some isolated with vancomycin's MIC up to 2 μg/mL. These findings alarm the risk of vancomycin treatment failure, as well as the possibility of VISA or VRSA developed with the widely use of vancomycin in the hospital.

P1-SP08

Characteristics of vancomycin intermediate *Staphylococcus aureus* (VISA) isolates in Korea, 2014

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Background: Vancomycin is one of the representative antibiotics for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Vancomycin-resistant *S. aureus* (VRSA) and vancomycin-intermediate *S. aureus* (VISA) have been a growing concern in many countries. In the present study, we investigated the prevalence and characteristics of VISA in Korea, 2014.

Methods: *S. aureus* strains were corrected from 93 hospitals participating in a nationwide surveillance program for VISA/VRSA in 2014. After screening on brain heart infusion agar with 4 mg/L vancomycin or automated testing systems, VISA strains were confirmed by vancomycin MIC values of agar, broth dilution or E-test according to CLSI guidelines (M100-S24, 2014). Molecular characterization was performed using toxin genes detection, SCC*mec* and *agr* typing, and pulsed-fields gel electrophoresis (PFGE).
Results: Eleven out of 28 screened isolates were identified as VISA strains with vancomycin MIC of 3 mg/L or more. The isolates were characterized as follows: enterotoxin genes of *sec*, *seg*, and *sei* with SCC*mec* II and agr II (9 isolates); *seg*, *sei* with SCC*mec* III and *agr* I (1 isolates); and *seg*, *sei* with SCC*mec* IV and *agr* I (1 isolates). In addition toxic shock syndrome toxin-1 (TSST-1) gene was detected in 8 isolates.
Conclusion: In 2014, 11 VISA strains were isolated in Korea, but no VRSA was detected. Most of VISA strains (81.8%) showed SCC*mec* II and *agr* II type patterns.

P1-SP09

Characterization of elastin-binding protein in ST5 methicillin-resistant *Staphylococcus aureus*

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Background: Methicillin-Resistant *Staphylococcus aureus* (MRSA) is one of the most frequent causes of infections, and the ST5 type is representative MRSA clone in Korea. Like many other *S.aureus* strains, MRSA strains produce a series of virulence factors. However, all the functions and mechanisms of the genes are not clear. In this study, we investigated the contribution of elastin-binding protein (*ebp*) gene to virulence using knockout system in the ST5 MRSA.
Methods: The *S.aureus-E.coli* shuttle vector (pKOR) was used for *ebp* gene knockout vector construction. The *ebp* knockout mutants of ST5 MRSA S3659 and S3670 strains were constructed by an allelic replacement methods; S3659Δ*ebp* and S3670Δ*ebp*. The virulence gene expression was analyzed by real time pcr, adhesion, invasion, cytotoxicity, binding to extracellular matrix components, and antimicrobial susceptibility of mutants were compared to those of parent strains.
Results: The gene expressions of *lgrA, icaA, sspA,* and *efb* were significantly increased in the *ebp* knockout strains. On the other hand, the knockout and the parent strains did not show any significant difference in the adhesion and invasion activity, and S3670Δ*ebp* strain showed lower cytotoxicity compared to the parent strain. The deletion of *ebp* in both knockout strains caused a significant reduction of ECM binding capacity including the elastin from human lung, cFN, pFN, and type IV collagen. The antimicrobial susceptibility of imipenem indicated that S3670 stain was resistant, but the S3670Δ*ebp* was susceptible.
Conclusions: The deletion of *ebp* gene in ST5 MRSA may affect the expression of some virulence genes and ECM binding capacity. It also influenced the cytotoxicity and the antimicrobial susceptibility against imipenem of the S3670Δ*ebp* strain.

P1-SP10

Prevalence and molecular characterization of methicillin-resistant *Staphylococcus aureus* from pigs and farm workers in Korea

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as zooonitic resistant bacteria. Furthermore incidence of MRSA in animals has increased in Korea recently. Therefore, the aims of this study were to investigate the prevalence and to characterize the MRSA isolates from pigs and farm workers in Korea.
Methods: Between February 2012 and May 2013, a total of 671 nasal cavity swab samples were collected from pigs and farm workers of 31 pig farms throughout Korea. The types of *mec* gene complex, toxin genes and host specific genes were determined by PCR. In addition, multilocus sequence typing and *spa*-typing were performed to determine the genetic relatedness of the MRSA strains.
Results: The prevalence of MRSA was 5.3% (31/580) in pigs and 17.6% (16/91) in farm workers, respectively. Highest prevalence (9.9%, 14/142) of MRSA was observed in weaned piglets among the age groups. Two different lineages were found among the 47 MRSA isolates from pigs and workers: 31 and 11 strains of livestock-associated type (ST398 or ST541/ *spa* t034 or t034 variant) and 0 and 5 strains of human-associated type (ST72/ *spa* t5440 or t664 or t148), respectively. All HA MRSA isolates carried enterotoxin, leukotoxin genes and/or host specific genes, whereas LA MRSA not. However, all LA MRSA isolates were multi-drug resistant, whereas HA types were susceptible or resistant to less than two antimicrobials. Furthermore, uncommon antimicrobial resistance genes such as *fexA*, *cfr*, and *tetL* were detected in LA MRSA isolates. Biofilms were formed by 42.9% (18/42) of LA MRSA isolates, whilst HA MRSA isolates not.
Conclusion: To our knowledge, this is the first report of LA MRSA in farm workers in Korea. This result suggests that MRSA in pigs may pose health risk especially for people who frequently come into contact with pigs**.**

P1-SP11

The different effect of concentration and contact time of chlorhexidine and triclosan on the growth of methicillin resistant *Staphylococcus aureus* (MRSA)

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Staphylococcal infection spread specially in hospital as nosocomial infection could be controlled by the use of proper antiseptic to medical staff. This study investigated inhibition effect of chlorhexidine and triclosan to *Methicillin Resistant Staphylococcus aureus* (MRSA) growth using various concentration and contact time. Each antiseptic and its concentration have been tested to 4 MRSA isolate i.e. MRSA I, II, III, IV for various contact time i.e. 30", 60", 90" and 120". Concentration of chlorhexidine were 0.25%, 0.5%, 1%, 2% and 4%. Concentration of triclosan were 0.125%, 0.25%, 0.5%, 1% and 2%. All treatments were replicated 4 times. Inhibition to each antiseptic compared to control group.
Statistical analyses mean of multivariate ANOVA continued to LSD showed significant different in all treatment ($p<0.01$). There was significantly different between concentrations. Significance different in contact time only happened between 30", 60" and 90". Result showed different inhibition effect of MRA growth by chlorhexidine compared to triclosan in various concentration and contact time. Antiseptic concentration and contact time were major influent to inhibit the growth of MRSA.
The conclusion of this investigation showed that 0.5% concentration of chlorhexidine, 0.25% concentration of triclosan and 90" contact time were the most effective concentration and contact time for inhibition *Methicillin Resistant Staphylococcus aurus* growth.
Keywords: chlorhexidine, triclosan, concentration, cantact time, MRSA

P1-SP12

MRSA in a newly-established healthcare centre: The UiTM experience

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major contributor of nosocomial infection. It limits treatment options, causes increased lengths of stay, costs, and mortality. We aimed to investigate trend of MRSA infection rates and its contributing cases in a newly-established, initially MRSA-free, healthcare facility. We retrospectively studied all *Staphylococcus aureus* cultured from patients in Faculty of Medicine UiTM over the first four years of operation. MRSA were determined as those resistant to cefoxitin by Kirby-Bauer and sensitive to vancomycin by E-Test methods. MRSA infection rates were expressed as cases per 1000 hospital admissions. MRSA were not isolated in 2011, 2012 and 2013 leading to infection rate of zero. Isolated cases eventually emerged in different months of 2014, resulting in infection rate of 1.1 per 1000 admission. All isolates were retrieved from wound swabs and showed antimicrobial susceptibility of hospital acquired MRSAs (HA-MRSA). Corresponding blood cultures were negative for MRSA. Half of these patients were positive for nasal swab MRSA performed upon their transfer-in from other hospitals. An MRSA career detected upon screening did not develop infection throughout admission. This centre was free of MRSA for the first three years of its establishment. The four isolated cases detected in 2014 were among transferred-in patients from other centres. All cases were surgical site infections, none of which developed bloodstream infections. MRSA surveillance of inpatients was helpful in predicting half of those, as well as identifying a silent carrier. This study demonstrated that active screening, along with other infection control procedures, was important in preventing initial spread in an MRSA-free hospital.

Year	2011	2012	2013	2014
Methicillin-resistant *Staphylococcus aureus* (MRSA) case	0	0	0	4
Total number of admission	276	859	2007	3669
MRSA infections rate (case per 1000 admission)	0	0	0	1.1

	Case 1	Case 2	Case 3	Case 4	Case 5
Month of 2014	July	August	September	November	December
Location	Ward 1	Ward 1	ICU	Ward 1	Ward 1
Infection	Surgical site	Surgical site	Surgical site	Nil	Surgical site
Pus swab culture	Positive	Positive	Positive	Nil	Positive
Blood culture	Negative	Negative	Negative	Nil	Negative
Nasal swab culture	Positive	Negative	Positive	Positive	Negative
Treatment	Vancomycin	Vancomycin	Vancomycin	Mupirocin	Vancomycin

P1-SP13

Genotype and diversity of virulence markers in *Staphylococcus aureus* isolated from wounds in Malaysian subjects

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Staphylococcus aureus is a potential causative agent for a variety of infectious diseases ranging from superficial skin infections to severe systemic infections. Our previous studies showed that *S. aureus* was the predominant microorganism in chronic wounds. However, correlation between the genotype of *S. aureus* and biofilm formation in wounds remains largely unknown. We genetically characterised 50 strains of *S. aureus* isolated from wounds for the frequency of 21 genes coding for microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), toxins and other virulence determinants by using high throughput real-time PCR. *S. aureus* strains were genotyped by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Real-time PCR showed that *fnb*, *ica, clf*, *eno* and *sdr* genes were found to be present in all isolates. The most prevalent virulence gene combinations (44%) were *fnb*, *fib*, *ica*, *clf*, *bbp*, *ebps*, *cna*, *sdr*, *eno*, *lukE*, *mecA*, *chp*, *hlg*. PFGE revealed 45 unique banding patterns that could be divided into 12 clusters based on 75% similarity. 30% of the isolates exhibited the B3 pulsotype. 23 sequence types (ST) obtained by MLST were assigned into 15 Clonal Complexes (CC) and 1 singletone. 3 novel STs were discovered and archived in the MLST database. The clustering of the isolates by PFGE was in agreement with MLST. PFGE however showed higher discriminatory power than MLST. Our results indicated that there was no strong correlation between the genes for biofilm formation with the genotype of *S. aureus*. Overall, this study provides an overview of the prevalence of virulence markers found in *S. aureus* in the wound setting.

P1-SP14

Prevalence of inducible clindamycin resistance in methicillin-resistant *Staphylococcus aureus* isolates in the Philippines

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Background: Increasing local rates of methicillin-resistance in *Staphylococcus aureus* causing both nosocomial and community associated infection, has led to increasing reliance on alternative antibiotic therapy such as clindamycin. This study was undertaken to evaluate the prevalence of inducible clindamycin resistance (iMLSB) among methicillin-resistant *S. aureus* isolates in the Philippines.

Methods: This was a prevalence survey on *S. aureus* clinical isolates from the Antimicrobial Resistance Surveillance (ARSP) sentinel hospitals over a 2-year period from January 2012 to December 2013. All identified *S. aureus* isolates had antibiotic susceptibility testing done using either the Kirby Bauer disc diffusion method or minimum inhibitory concentration determination. These isolates were also screened for inducible clindamycin resistance by the double disc diffusion assay (D-test).

Results: There were 4,272 *S.aureus* isolates reported from 2012 to 2013 in the ARSP with 58.6% (2,503 isolates) testing as methicillin resistant (MRSA). Among these MRSA isolates, 9.5% (239 isolates) were resistant *in vitro* to both erythromycin and clindamycin and were considered to have the MLSB constitutive resistance phenotype (cMLSB); while 0.2% (4 isolates) tested resistant to erythromycin, susceptible to clindamycin but demonstrated a positive D test and were considered to be positive for inducible clindamycin resistance (iMLSB).

Conclusion: The low prevalence of inducible clindamycin-resistance in methicillin-resistant *S.aureus* isolates locally supports the use of clindamycin in treatment of MRSA infections but we recommend that the D test be included in the routine antibiotic susceptibility testing to help prevent its inappropriate use.

P1-SP15

SCC*mec* IX-ST9 methicillin-resistant *Staphylococcus* from pig farms in Thailand

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Introduction: Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) of Clonal Complex (CC) 398 was reported in Europe, whereas CC9 MRSA was mostly found in Asia. We therefore aimed to detect MRSA from pig farms in northeast of Thailand.
Methods: A total of 257 nasal swabs (159 samples from pigs and 98 from pig farm workers) were collected from 3 pig farms in northeast of Thailand during 2010-2011. MRSA isolates were confirmed by PCR for *femA* and *mecA* genes. The minimum inhibitory concentrations of eight antimicrobials, vancomycin, cefazolin, olfloxacin, tetracycline, erythromycin, oxacillin, cefoxitin and gentamicin, were tested by agar dilution method. Virulence genes, Valentine leukocidin toxin (*lukSF-PV*), toxic shock syndrome toxin-1 (*tst*) and α-hemolysin (*hla*) were detected by PCR. Strain typing was performed by SCC*mec*, *agr*, *spa*, and multilocus sequence typing.
Results: Four MRSA were isolated, 3 from workers and one from pig. All MRSA were resistant to oxacillin, gentamicin, erythromycin, tetracycline, cefazolin, and carried *hla* only. Two MRSA from human carried SCC*mec*II-ST764-*agr*II, whereas the two remaining MRSA (each from human and pig) contained SCC*mec* IX-ST9-*agr*II. Interestingly, methicillin-resistant coagulase negative *Staphylococcus* isolates carrying SCC*mec* IX were also obtained from 6 workers and 4 pigs.
Conclusions: This study suggests that there is a distribution of SCC*mec* IX element among *Staphylococcus* in pigs and pig farm workers and pigs may be a reservoir of MRSA in the community.

P1-SP16

Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* circulating in the Russian Federation

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Background: Spread of MRSA in hospitals, is a serious threat to the healthcare system in Russia, the prevalence of MRSA varies in different regions, from 0 to 80%. Here we are present the data on the molecular epidemiology of MRSA.
Methods: Non-repetitive MRSA isolates were recovered from patients with different staphylococcal infections and healthy carriers in 13 regions of Russia (from Krasnoyarsk to Saint Petersburg) during 2011 - 2014. Multilocus sequence typing (MLST) and *spa*-typing were performed as described at www.mlst.net and www.spaserver.ridom.de, respectively. PCR was used for SCC*mec*, and *agr* typing, as well as for resistance and virulence genes detection.
Results: Among 470 isolates under the study 13 different sequence-types (ST's), 33 *spa* variants and 12 types and subtypes of SCCmec were detected. The genotypes ST8-t008/t024-SCC*mec*IVce and ST239-t032/t037-SCC*mec* III were the most prevalent in all geographical regions, 47% and 29% respectively. All isolates of these genotypes belonged to *agr* I group. Isolates of genetic lineage ST8 were recovered from healthy nasal carriers and from patients with invasive infections, while ST239 isolates were recovered only from patients with invasive diseases. In ST8 isolates macrolides and tetracyclines resistance were mediated by *erm*C and *tet*K genes; while in ST239 –by *erm*A and *tet*M.
In Moscow and Saint Petersburg ST228-t041-SCCmec IA (*agr* II) and ST239-t632-SCCmec III were detected in 7% of cases each. In Krasnoyarsk the lineage ST239 – t032/t037 –SCCmec III with *tsst* toxin gene was detected. Isolates belonging to ST22-t032-IVH, ST398-t011-IVA, ST764-t042-II, ST5 and ST97 were detected in less than 1% of cases each. In all isolates *lukFS*, *seb* and ACME-complex genes were lacking.
Conclusion: MRSA population in Russia is highly clonal and represented by HA-MRSA, CA-MRSA were not detected.

P1-SP17

Comparison of PVL-positive MRSA and MSSA isolated from Chinese children with SSTIs

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Background: PVL is an important virulence gene contributing to staphylococcus aureus. Information on PVL-positive staphylococcus aureus in Chinese children is limited. This study aims to compare demographics, molecular characteristics and antibiotic susceptibility of PVL-positive MRSA and MSSA isolated from Chinese children with SSTIs.
Methods: Staphylococcus aureus were collected and analyzed Panton-Valentine leukocidin gene (*pvl*) gene detection. Then PVL-positive isolates which causing skin and soft-tissue infections (SSTIs) were analyzed by MLST typing, SCC*mec* typing and spa typing.
Results: 35.7% CA-MRSA, 19.8% HA-MRSA and 32.6% MSSA were PVL-positive. Among PVL-positive MRSA, 7 kinds of ST types were detected and ST59 are the dominant one; 3 kinds of SCCmec were detected and SCCmec IV were the prevalent one; 11 kinds of spa types were determined and t437 were the most common one; The most prevalent clone is ST59-SCCmecIV-t437 (33.3%, 11/33). Among PVL-positive MSSA, 19 kinds of ST types were found, ST398 (34.1%, 30/88) are the most common one; there are no dominant one among 39 spa types found; no prevalent clone are found and ST398-t034 are the most common one (14.8%, 13/88). PVL-positive MRSA has significant higher resistance to ten kinds of antibiotics detected than PVL-positive MSSA. Interestingly, two isolates resistance to linezolid are firstly found in Chinese children with PVL-positive MSSA other than PVL-positive MRSA.
Conclusion: PVL carriage in Chinese children with staphylococcus aureus infection is high. ST59-SCCmecIV-t437 were the most prevalent clone in PVL-positive MRSA isolated from Chinese children with SSTIs. And there were no prevalent clone in PVL-positive MSSA. PVL-positive MSSA have high antibiotics resistance in Chinese children. More attention should be taken to infections caused by MSSA.

P1-SP18

Molecular characterization of methicillin-susceptible *Staphylococcus aureus* blood isolates with high vancomycin MICs

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Background: High minimum inhibitory concentration (MIC) of vancomycin in methicillin-resistant *Staphylococcus aureus*

(MRSA) has been considered as one of the causes of vancomycin treatment failure. Among methicillin-susceptible *S. aureus* (MSSA), however, strains with high vancomycin MICs are often found. We characterized 71 MSSA blood isolates with high vancomycin MICs.
Methods: Vancomycin susceptible MSSA with vancomycin MIC ≥1 mg/L was selected among the *S. aureus* collection from the previous nationwide surveillance study on bacteremia in Korea during the periods 2006-2007 and 2012-2013. MSSA isolates with vancomycin MIC <1 mg/L were randomly selected in the ratio of 1:1 as the control. Multilocus sequence typing (MLST) was performed.
Results: Among 242MSSA isolates, a total of 71 isolates (29.3%) with vancomycin MIC ≥1 mg/L were identified. MLST showed 19 different sequence types. The predominant sequence types were ST72 (15.5%) and ST6 (14.1%), followed by ST1 (12.7%) and ST30 (12.7%). ST188 accounted for 7.0%. In control group, 21 different sequence types were identified with distribution of ST30 (19.7%), ST188 (14.1%), ST72 (11.3%), and ST5 (9.9%). ST1 and ST6 accounted for 5.6% and 2.8%, respectively.
Conclusion: This study shows that strains with high vancomycin MICs are identified in a substantial proportion of MSSA blood isolates. ST72, ST6, and ST1 were more frequently found in MSSA with high vancomycin MICs compared to MSSA with low vancomycin MICs of which ST30 and ST199 were predominant. Further molecular characterization of those strains will be followed.

P1-SP19

Frequency and distribution of single nucleotide polymorphisms (SNPs) within *mprF* in methicillin-resistant *Staphylococcus aureus* clinical isolates

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MprF is a lysyl-phosphotidylglycerol (L-PG) synthase which transfers positively-charged lysine molecules from lysyl-tRNA and adds them to phosphotidylglycerol (PG) within the *S. aureus* cell membrane (CM). In addition, MprF functions as an inner-to-outer CM translocase for L-PG. Single nucleotide polymorphisms (SNPs) within the *mprF* ORF have been associated with a gain-in-function phenotype for daptomycin resistance (DAP-R) in *S. aureus*. In this investigation, we used 22 DAP-S and DAP-R paired clinical MRSA strains to assess : i) the frequencies and distribution of putative *mprF* gain-in-function SNPs, ii) their relationships to both DAP-R and 'cross-resistance' to tPMPs, and iii) the impact of *mrpF* mutations on surface positive charge phenotype resulted from modifications of PL contents. All of the *mprF* SNPs identified in our DAP-R strains were clustered within the two MprF loci : the putative bifunctional domain or the C-terminal synthase domain. Moreover, we were able to correlate the presence and location of *mprF* SNPs in DAP-R strains with HDP cross-resistane, surface positive charge, and L-PG profiles. Althogh DAP-R strains with *mprF* SNPs in the bifunctional domain showed higher resistance to tPMPs than DAP-R strains with SNPs in the synthase domain, this relationship was not observed in surface positive charge assays. These results suggested that both charge-related and –unrelated mechanisms are involved in DAP and HDP resistances in MRSA strains.

P1-ST01

The sensitivity to cefaclor of *S. pneumoniae*, *H. influenzae* and *S. pyogenes* isolated from respiratory tract of patients with acute respiratory infection

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Background: Currently, doctors have to deal with the challenges of treatment failure when using ampicillin, co-trimoxazol, or the antibiotic erythromycin as initial antibiotic to the acute respiratory infections because *S. pneumoniae* and *H. influenzae* which are the main pathogens of the diseases are resistant to these antibiotics with the very high ratio. Therefore, finding an initial antibiotic to treat the acute respiratory infections in the community is a necessity.
Aims of the study: Study on the sensitivity to cefaclor through the MIC parameter of *S. pneumoniae*, *H. influenzae* and *S. pyogenes* isolated in Vietnam from patients with acute respiratory infections and from that it may recommend the physicians may consider the use of first choice antibiotic for the treatment of the acute respiratory infections in community.
Objects and methods: Subjects of the study were *S. pneumoniae*, *H. influenzae*, and *S. pyogenes* isolates from specimens obtained from the respiratory tract of patients with acute respiratory infections who were hospitalized or examined in hospital Nguyen Tri Phuong from 1/2012 to 12/2013. Research methodology is carried-out the susceptibility of cefaclor by micro-dilution according to the CLSI standards for MIC determination. Besides that, the β-lactamase detection using nitrocefin as chromogenic substrate were also carried-out to *H. influenzae* according to the suppliers instruction. The received results are recorded in the Excel file and analyzed by the descriptive statistics method.
Results: There were 200 *S. pneumoniae*, 200 *H. influenzae*, and 32 *S. pyogenes* isolates were collected and studied. The results of the cefaclor MIC shows that the ratio of *S. pneumoniae* resistant to Cefaclor is 89% (based on the MIC resistant breaking point 4 μg/ml), while the ratio of *H. influenzae* resistant to cefaclor is 13% (based on the MIC resistant breaking point 32 μg/ml). *S. pyogenes* were highly sensitive to cefaclor with 100% of cefalor MIC was 0.25 μg/ml or lower. The MIC distribution to cefaclor of *S. pneumoniae* cefaclor tend to fall on the higher MIC values (32, 64 and 128 μg/ml), whereas the distribution of cefaclor MIC to *H. influenzae* gathering more among sensitive MIC (0.25, 0.5, 2, 4 and 8 μg/ml). The study also showed that 41% of *H. influenzae* producing β-lactamase and both producing and non-producing were sensitive to cefaclor with high ratio. In addition, the differences in the ratio of cefaclor sensitive of the isolates originated from adults or children, from the lower respiratory or the upper respiratory also recorded and analyzed.
Discussion: The obtained results showed that cefaclor should not be recommended as the first choice antibiotic for the treatment of acute respiratory infections caused by *S. pneumoniae* because of the high ratio of resistance of this pathogen to cefaclor; in contrarely, the doctors should consider cefaclor among the first choice antibiotic for the treatment of acute respiratory infection caused by *H. influenzae* due to its high ratio of sensitive to this antibiotic. Another reason for the recommendation of cefaclor to the treatment of *H. influenzae* infection is its support to the immune effect by increasing the efficiency of the phagocytes to kill the bacteria as reported in many previous studies, and with this effect, cefaclor may reach 100% in-vivo sensitivity to *H. influenzae*. For the *S. pyogenes*, study results showed that 100% of collected isolates in the study were sensitive to cefaclor with very low MICs ≤0.25 μg/ml, therefore cefaclor should be considered as the very effective antibiotic for the treatment of acute respiratory infections due to *S. pyogenes* such as purulent pharyngitis or purulent tonsillitis.

Conclusion: The studied results showed that cefaclor should be selected as the effective antibiotics to treat the acute respiratory infections caused by *H. influenzae* or *S. pyogenes* due to the high ratio sensitivity to cefaclor of these pathogens. For the *S. pneumoniae* pathogen, because of its high ratio resistance as 89% to cefaclor, the doctors could only consider to use these antibiotics in the treatment if the susceptibility results show that the MIC of the isolate to the cefaclor is not over the MIC resistant breakpoint 4 μg/ml.

P1-ST02

Serotype and genotype distribution of macrolide resistant *Streptococcus pneumonia* isolates from adult patients in Korea

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Background: *Streptococcus pneumonia* is a major pathogen to cause severe infections. Macrolides are frequently prescribed in case of treatment failure with beta-lactam therapy. Therefore, it is important to understand the current status of macrolide resistance in pneumococcal isolates. This study was performed to investigate changes in serotypes and genotypes of macrolide resistant *S. pneumoniae* in Korea.
Methods: The Korean Network for Study on Infectious Diseases (KONSID) performed a prospective surveillance study of 192 *S. pneumoniae* collected from adult patients (≥50 years old) with pneumococcal infections in Korea from 2012 to 2014. Serotypes of *S. pneumoniae* isolates were determined by the capsular Quellung method and in vitro antimicrobial susceptibility tests were performed by broth microdilution method. Erythromycin-resistant isolates were subjected to PCR analysis to detect the *erm*(B) and *mef*(A) genes. In addition, serotypes and genotypes of erythromycin-resistant isolates were compared with those of147 erythromycin-resistant isolates (147/198, 74.2%) collected during 2008-2009 in Korea.
Results: A total of 149 *S. pneumonia* isolates were resistant to erythromycin (77.6%) and prevalence of azithromycin and clarithromycin resistance were 77.6% and 75.5%, respectively. The level of macrolide resistance has increased with MIC_{50} of 128 μg/ml and MIC_{90} of >64 μg/ml in 2012-2014 from 32 μg/ml and 128 μg/ml, respectively, in 2008-2009. Of 149 erythromycin-resistant isolates, 29.5% carried only *erm*(B) and 10.7% carried only *mef*(A), whereas 59.1% (88/149) contained both *erm*(B) and *mef*(A). Among the isolates collected in 2008-2009, 35.4% (52/147) contained both genes. The most prevalent serotypes among macrolide resistant isolates were 19A (12.8%), followed by 11A (12.1%), 19F (9.4%), and 35B (8.7%) in 2012-2014 while those were 19F (13.6%), 19A (11.6%), 9V (10.2%), and 6A (8.2%) in 2008-2009.
Conclusion: This study showed the persistently high prevalence of macrolide resistance and increasing number of pneumococci carrying both *erm*(B) and *mef*(A) in Korea. Given the current epidemiology of macrolide resistance, an empirical use of macrolides alone may not be an appropriate choice for the treatment of pneumococcal infection in adult patients in Korea. Therefore, continued surveillance of pneumococcal epidemiology of macrolide resistance would be necessary.

P1-ST03

Changes in serotype distribution of *Streptococcus pneumonia* isolates from adult patients with invasive pneumococcal disease or pneumonia in Korea

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Background: The introduction of pneumococcal conjugate vaccine (PCV) has resulted in changes in epidemiology of pneumococcal diseases. This study was performed to investigate the changes in serotype distribution of *Streptococcus pneumonia* and to understand the impact of PCV on the epidemiology of pneumococci in Korea.
Methods: The Korean Network for Study on Infectious Diseases (KONSID) performed a prospective surveillance study on *S. pneumoniae* collected from adult patients (≥50 years old) with invasive pneumococcal disease or pneumonia in Korea in 2008-2009 and in 2012-2014. Serotypes of *S. pneumoniae* isolates were determined by the capsular Quellung method.
Results: A total of 198 and 192 non-duplicate *S. pneumonia* isolates were prospectively collected in 2008-2009 and in 2012-2014, respectively. The primary specimen sources are sputum (73.7% and 50.6% in 2008-2009 and in 2012-2014, respectively) and blood (20.2% and 40.2%, respectively). Major serotypes of pneumococcal isolates collected in 2012-2014were 19A (10.4%), 3 (9.4%), 11A (9.4%), 19F (7.3%), and 35B (7.3%) while major serotypes were 19F (11.1%) and 3 (11.1%), followed by 19A (9.6%) and 6A (6.6%) in 2008-2009. The proportion of vaccine serotypes were 16.2%, 16.2%, and 39.6% for PCV7, PCV10, and PCV13, respectively, in 2012-2014. These percentages were much lower than the rates of pneumococcal isolates collected in 2008-2009 (37.4%, 39.9%, and 67.2%, respectively).
Conclusion: This study showed the dramatic changes in the distribution of serotypes after the introduction of PCV vaccination in Korea, with the remarkable increase of non-vaccine serotypes such as 11A and 35B. These data emphasize the need for continued surveillance of pneumococcal epidemiology in the post-PCV13 era.

P1-ST04

Antimicrobial resistance of *Streptococcus pneumoniae* isolates from adult patients with invasive pneumococcal disease or pneumoniae in Korea

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Background: The disease burden of *Streptococcus pneumoniae* has increased due to widespread emergence of antimicrobial resistance in many countries despite the introduction of the pneumococcal conjugate vaccine (PCV). This study was performed to investigate the changes in antimicrobial susceptibility of *S.pneumoniae* and to understand the impact of PCV on the epidemiology of pneumococci in Korea.

Methods: The Korean Network for Study on Infectious Diseases (KONSID) performed a prospective surveillance study on *S. pneumoniae* collected from adult patients (≥50 years old) with pneumococcal infections in Korea in 2008-2009 and in 2012-2014. In vitro antimicrobial susceptibility tests were performed by broth microdilution method according to the guidelines of CLSI against 16antimicrobial agents.
Results: A total of 198 and 192 non-duplicate *S. pneumonia* isolates were prospectively collected in 2008-2009 and in 2012-2014, respectively. The prevalence rate of penicillin-nonsusceptible pneumococci (PNSP; MIC ≥4 μg/ml) were 2.5% and 2.1% in 2008-2009 and in 2012-2014, respectively. If the previous penicillin susceptibility breakpoints (MIC ≥2 μg/ml) were applied, penicillin resistance rates were 15.2% in 2008-2009 and 25.5% in 2012-2014. Macrolide resistance was very prevalent (74.2% and 77.6% in 2008-2009 and in 2012-2014, respectively). Multidrug resistance (MDR) was observed in 56.6% and 58.3% of isolates in 2008-2009 and in 2012-2014, respectively.
Conclusion: This study showed a persistently high prevalence of macrolide resistance and MDR in pneumococcal isolates. Given the high prevalence of resistance and its clinical impact, continued surveillance of pneumococcal epidemiology is strongly warranted in Korea.

P1-ST05

Clinical characteristics and antimicrobial resistance of non-vaccine serotype *Streptococcus pneumoniae* in Korea: a multicenter surveillance study

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Background: The purpose of this study was to determine clinical characteristics of systemic pneumococcal infections by non-vaccine serotypes (NVT).
Methods: Study subjects included adult patients with non-invasive pneumococcal pneumonia (≥65 years) and invasive pneumococcal disease (IPD) (≥18 years). Clinical data and pneumococcal isolates were collected from 20 participating hospitals in Korea from 2013 to 2014. Clinical characteristics (invasiveness, clinical severity, outcomes, antibiotic resistance) were compared between the NVT and the VT. Pneumococcal serotypes were determined by the immunoassay. Antibiotic susceptibility was taken by MicroScan.
Results: A total of 439 cases were enrolled; the VT (n = 253) and the NVT (n = 186). Of 118 IPD, 50 cases were from the NVT with 39.2% of case fatality rate, In multivariate analyses, IPD other than bacteremia pneumonia, primary bacteremia, empyema and meningitis were significantly associated with the NVT (OR, 5.41; 95% CI, 1.13-25.64; p=0.034). Interestingly, non-susceptibilities to either CEC or CXM(OR, 15.94; 95% CI, 7.31-34.78; p<0.001) and CHL (OR, 3.61; 95% CI, 1.92-6.80; p<0.001) were significantly higher in the NVT, whereas those to AMC (OR, 3.57; 95% CI, 1.78-7.14; p<0.001), TMP/SMX (OR, 4.05; 95% CI, 2.14-7.65; p<0.001) and TET (OR, 3.10; 95% CI, 1.52-6.30; p=0.002) were significantly higher in the VT.
Conclusion: This study indicates that invasiveness and mortality with the NVT are comparable to the VT. Increasing IPD by the NVT is emerging challenges for control of pneumococcal diseases.

P1-ST06

Increase of antimicrobial resistant *Streptococcus pneumoniae* serotype 11A in Korea during 2008-2013

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Background: Since the introduction of the pneumococcal conjugate vaccine in Korea, a significant decrease in invasive pneumococcal disease among children has been reported. However, the proportion of invasive diseases caused by non-vaccine serotype has increased. Among non-vaccine serotypes, serotype 11A was highly prevalent in Korea. In addition, six extensively drug-resistant (XDR) pneumococcal isolates, which were all serotype 11A but one isolate, were recently reported. We investigated the prevalence of *Streptococcus pneumonia* serotype 11A isolates in a Korean tertiary-care hospital during 2008-2013.
Methods: A total of 820 non-duplicate clinical isolates of pneumococci were collected in 2008 to 2013 from a tertiary hospital. Serotype was determined by the capsular Quellung method, and in vitro susceptibility testing was performed by broth microdilution method. Multilocus sequence typing was performed to determine the genotypes of the *S. pneumoniae* isolates. The *erm*(B) and *mef*(A) genes in erythromycin-resistant isolates were also detected using the duplex PCR method.
Results: We identified 54 *S. pneumoniae* serotype 11A isolates (6.6%) during 2008-2013.During this period, the proportion of serotype 11A isolates has increased from 1.9% (in 2008) to 14.3% (in 2013). Most *S. pneumoniae* serotype 11A isolates (79.6%)were multidrug resistant(MDR), and 18.6% of MDR isolates showed XDR phenotype. Most of serotype 11A isolates belonged to ST166 and its single- or double locus variants including ST8279, an XDR clone (42 isolates, 77.8%). Among erythromycin-resistant pneumococci, *erm*(B) gene (75.0%) was predominantly detected.
Conclusion: We identified increase of *S. pneumoniae* serotype 11A after the introduction of pneumococcal conjugate vaccine in Korea. Clonal expansion of a certain clone (CC166) was identified. Particularly, an XDR clone (ST8279) was also shown to be serotype 11A, and would be a significant therapeutic challenge.

P1-ST07

***In vitro* activity of tedizolid phosphate against multidrug resistant *Streptococcus pneumoniae* isolates from nine Asian countries**

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Background: Tedizolid phosphate is a second-generation oxazolidinone prodrug that is potential activity against a wide range of gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus*, penicillin-resistant streptococci, and vancomycin-resistant enterococci. In this study, we evaluated the in vitro activity of tedizolid and other comparator agents against *Streptococcus pneumoniae* isolates including levofloxacin-resistant or multidrug-resistant (MDR) isolates.
Methods: A total of 105 non-duplicate clinical isolates of pneumococci were collected in 2008 to 2009 from 9 Asian areas, including Korea, Taiwan, Thailand, Hong Kong, Vietnam,

Malaysia, Philippines, Saudi Arabia, and Sri Lanka. Serotypes of *S. pneumoniae* isolates were determined by the capsular Quellung method, and in vitro susceptibility testing for 12 antimicrobial agents was performed by broth microdilution method.
Results: Most *S. pneumoniae* isolates showed high resistance rates to tetracycline (92.4%), erythromycin (88.6%), clindamycin (73.3%), cefuroxime (67.6%), trimethoprim-sulfamethoxazole (70.5%) and levofloxacin (58.1%). Of the 105 *S. pneumoniae* isolates, 97.1% were MDR pneumococci. All isolates tested were inhibited at a tedizolid MIC value of ≤0.25 μg/ml (ranged from ≤0.03 μg/ml to 0.25 μg/ml). The MIC_{50} and MIC_{90} of tedizolid against MDR pneumococci were both 0.25 μg/ml, while $MIC_{50}$and MIC_{90} of linezolid were 0.5 μg/ml and 1 μg/ml, respectively. These results were consistent over Asian geographic regions. In addition, tedizolid maintained the activity against *S. pneumoniae* regardless of the levofloxacin-resistant phenotype of the isolates.
Conclusion: This study confirmed the potent in vitro activity of tedizolid against pathogenic MDR pneumococci, showing 4-fold-greater potency in comparison with linezolid. Tedizolid phosphate may be a reasonable alternative to linezolid for treating MDR pneumococci.

P1-ST08
High prevalence of multidrug resistant clone in levofloxacin non-susceptible *Streptococcus pneumoniae* isolates in Korea during 2008-2012
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Background: The increasing pneumococcal resistance to levofloxacin has become a major concern. We investigated the prevalence and genetic characteristics of levofloxacin non-susceptible *Streptococcus pneumonia* clinical isolates in Korea.
Method: A total of 42 levofloxacin non-susceptible *S. pneumoniae* isolates collected from multi-hospital network from 2008 to 2012 were analyzed for serotype and antimicrobial susceptibilities to 19 antimicrobial agents and quinolone resistance-determining region (QRDR) mutation. Multilocus sequence typing (MLST) was also performed to investigate the genetic relatedness among levofloxacin non-susceptible *S. pneumoniae* isolates.
Result: All levofloxacin non-susceptible *S. pneumoniae* isolates (MIC, ≥4mg/L) exhibited the multidrug resistant (MDR) phenotype. Among these MDR isolates, 7 isolates (16.7%) were extensively drug-resistant (XDR), defined as non-susceptible to at least one agent in all classes but vancomycin and linezolid. Serotype 11A (13 isolates, 33.3%) was most frequently found, followed by 19F (6 isolates, 14.3%) and 6D (3 isolates, 7.1%), respectively. Most predominant amino acid substitutions in the QRDRs of levofloxacin non-susceptible *S. pneumonia* were at Ser81-Phe in *GyrA* (33 isolates, 78.6%), Ser79-Phe and Lys137-Asn in *parC* (29 isolates, 69.0% and 27 isolates, 64.3%, respectively) and Ile460-Val (40 isolates, 95%) in *ParE* gene. Most of levofloxacin non-susceptible *S. pneumoniae* isolates belonged to sequence type (ST) 8279 and its single-locus variants (15 isolates, 35.7%), which is a double-locus variant of ST156 closely related to the pneumococcal Spain9V-3 international clone.
Conclusion: All levofloxacin non-susceptible *S. pneumoniae* isolates in Korea exhibited high prevalence of MDR, even XDR phenotype, which indicates the use of levofloxacin should be more judicious in adult patients with community-acquired pneumonia. The high prevalence of non-vaccine types in levofloxacin non-susceptible *S. pneumoniae* isolates, especially such as predominant serotype 11A in XDR pneumococci, could be significant therapeutic challenges. More information on the emergence and spread of these levofloxacin non-susceptible *S. pneumoniae* isolates would be necessary in order to prevent its spread.

P1-ST09
Serotype distribution, antimicrobial resistance and molecular characterization of invasive Group B Streptococcus isolates recovered from Chinese neonates
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Background: Group B Streptococcus (GBS) is an important neonatal pathogen associated with high morbidity and mortality in developed countries. However, data describing neonatal GBS disease in developing countries, particularly in Asia, are largely incomplete. The aim of this study was to determine serotype distribution, antimicrobial resistance, and molecular characteristics of invasive GBS isolates recovered from Chinese neonates.
Methods: From 2008 to 2013, 40 GBS isolates were recovered from infected neonates less than 3 months of age. All isolates were identified with the CAMP test and commercially available techniques. Serotyping was performed by latex agglutination and antibiotic susceptibility was tested with E-test strips and the disk diffusion method. Multilocus sequence types and erythromycin resistance gene detection (*ermB* and *mefA*) were performed by polymerase chain reaction.
Results: Our serotypes were identified. Serotype III (85%) was the most prevalent, followed by Ia (7.5%), Ib (5%) and V (2.5%). All isolates were sensitive to penicillin, ceftriaxone and levofloxacin. However, resistance to erythromycin (92.5%), clindamycin (87.5%), and tetracycline (100%) was observed. Among erythromycin-resistant isolates, 73.0% carried the *ermB* gene alone, 5.4% carried the *mefA* gene alone, and 21.6% expressed both *ermB* and *mefA* genes. A total of 7 sequence types (STs) were identified; the most prevalent was ST17, accounting for 80% of all isolates. Further, serotype III isolates contained ST17 (94.2%), ST19 (2.9%), and ST650 (2.9%).
Conclusion: Serotype distribution, antimicrobial susceptibility and sequence type characterization in Asia and in other global regions may improve prevention and treatment of neonatal GBS infections.

P1-ST10
Assessing the rising cases of methicillin-resistant *Staphylococcus aureus*: hospital and community-associated cases
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Methicillin-resistant *Staphylococcus aureus* (MRSA) has since become a major cause of illness and death in our healthcare setting. Risk factors for HA-MRSA include hospitalization, older age, invasive devices, and residence in long-term care facility,

including exposure to antimicrobial agents. HA-MRSA isolates are often resistant to several antimicrobial drug classes in addition to beta-lactams. The CA-MRSA infections usually affects young, healthy persons and associated with sharing towels or athletic equipment, participating in contact sports, living in unsanitary and crowded areas, using illegal intravenous drugs.
Directions were given out for clinical microbiology laboratories to submit invasive isolates of MRSA to our unit, where we perform antimicrobial drug susceptibility tests on all isolates and characterize all isolates that were resistant to <3 non-beta-lactam antimicrobial drug classes. Most isolates were obtained from blood cultures.
The full model for predicting invasive infection with CA-MRSA compared with HA-MRSA included age, seasonality, and hospital exposure, plus specimen type. The only significant predictors of CA-MRSA infection compared with HA-MRSA were age <69 years, which was associated with increased risk ([OR] 5.1, 95% [CI] 2.06-12.64), and hospital exposure (OR 0.07, 95% CI 0.01-0.51), which was associated with decreased risk. Most patients were hospitalized for their infections and the proportion of patients admitted to intensive care units did not vary by strain. Patients infected by MRSA were younger than those infected by other strains.
The number of invasive MRSA infections reported and the number of invasive infections caused by CA-MRSA is on the increase. The increase of CA-MRSA poses a unique public health threat. It is now clear that CA-MRSA no longer causes only SSTIs but now causes an increased proportion of invasive infections in a rural state.

P1-ST11

Differential expression of neuraminidase genes (NanA and NanB) in A549 human lung epithelial cells infected with pneumococcal strains of various serotypes

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Background: *Streptococcus pneumoniae* is the most common bacterial respiratory pathogen that can lead to invasive diseases such as pneumonia, bacteremia, and meningitis. Several virulence determinants have been identified in *S.pneumoniae*. In the present study we investigated the expression of neuraminidase genes (NanA and NanB) after interaction of A549 human lung epithelial cells with pneumococcal serotypes.
Methods: Six different serotype of *S.pneumoniae* were tested (1, 3, 5, 19F, 23F, and 14). A549 human lung epithelial cells were pneumococcal strains of different serotypes and incubated for 3 hours. Bacterial RNA was then harvested from the infection model. Morphological changes of A549 cells upon infection for were observed using inverted microscope. NanA and NanB genes expression were analyzed by quantitative real-time PCR (qRT-PCR) using aiTaq Universal SYBR Green One Step kit (Bio-Rad). Expression data were normalized against housekeeping gene, *gyr*A. Fold changes were determined using the $2^{-\Delta\Delta Ct}$ method. Nonparametric Mann-Whitney U test was used for statistical analysis. All analysis was performed using IBM SPSS Statistics 20.
Results: Six serotypes used in this study are the vaccine-type**.** Adherence and invasion of pneumococcal isolates was observed on the surface of the A549 cells. NanA and NanB are important in colonization for pneumococcal adherence in the respiratory tract and bloodstream. The high levels of diversity in neuraminidase gene cause dissimilarity present across different serotypes, which may affect the potential use of vaccine. NanB gene expression showed a statistically significant difference across different serotypes. NanA gene was significantly upregulated in serotypes 19F but significantly downregulated in serotype 3 and 14. There is no significant upregulation in serotype 1, 5 and 23F. Meanwhile, gene NanB was significantly upregulated in serotype 14 and significantly downregulated in serotype 23F. Whereas the other serotype; 3, 5, 19F showed an upregulation of NanB gene except serotype 1 showed a downregulation but at an insignificant level.
Conclusions: These results indicate that differential expression occur in pneumococcal strains of different serotypes. These might give a better understanding of host pathogen interaction and development of target vaccine.

P1-ST12

Serogroup distribution and antimicrobial resistance patterns of *Streptococcus pneumoniae* in the Philippines 2004-2011

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Background: Data on prevailing pneumococcal serogroups and resistance patterns guide both vaccine and antibiotic recommendations. This study aims to provide an estimate of the serogroup distribution and antimicrobial resistance patterns of *Streptococcus pneumoniae* causing invasive infections in the Philippines.
Methods: This was a prevalence survey on *S. pneumoniae* isolates referred to the Antimicrobial Resistance Surveillance Reference Laboratory from 2004 to 2011. The invasive *S. pneumoniae* isolates were identified, serogrouped through slide agglutination and tested for antimicrobial susceptibility using either the Kirby Bauer disk diffusion or MIC E-test method.
Results: A total of 195 isolates of *S. pneumonia* from CSF and blood were referred to the reference laboratory from 2004 to 2011. The most prevailing serogroups in this study were 1, 2, 3, 4, 5, 6, 12, and 23 (72.3%); while 7isolates (3.59%) were non-typable. Antimicrobial susceptibility results showed resistance to penicillin (2.5%), erythromycin (0.5%), co-trimoxazole (0.2%), and levofloxacin (1.03%) whereas 2% were multi-drug resistant. All of the isolates were observed to be susceptible to ceftriaxone. The PCV13 vaccine covered 73.3% of the sergroups identified in this study.
Conclusion: The study shows that prevalent serogroups of *S. pneumoniae* causing invasive diseases in the Philippines are mostly susceptible to antibiotics including penicillin. Of the available PCV vaccines locally, the PCV13 covered most of the serogroups identified in this study. The serogroup distribution and resistance patterns of *S. pneumoniae* will continue to evolve with better implementation of a vaccine program. This underscores the importance of continued surveillance of emerging serogroups, resistance patterns and evaluates public health interventions.

P1-ST13

Erythromycin and clindamycin resistance in *Streptococcus pyogenes* over the last 10 years in Korea

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Background: Although penicillin is the drug of choice for the eradication of *Streptococcus pyogenes* in the throat, macrolides are adequate alternative drugs, particularly for patients who are allergic to penicillin. Many reports have suggested that antibiotic

resistance rates depend on the consumption of antibiotics in the community due to selective pressure.
Methods: We reviewed recent national and international data for erythromycin and clindamycin resistance. Macrolide resistance phenotypes were compared, and the association of *emm* genotypes with antibiotic resistance was investigated. The correlation between erythromycin resistance and macrolide consumption was analyzed. Macrolide resistance were classified by cMLS, iMLS, and M according to the resistance pattern for erythromycin and clindamycin double disk. *emm* genotype was determined by DNA sequencing and BLAST program.
Results: Resistance to erythromycin was highest (51%) in 2002, and then dropped dramatically (to 10%) in 2004. It remained at less than 10% until now. In contrast, the consumption of macrolides, particularly new macrolides such as azithromycin, roxithromycin, and clarithromycin, has increased continuously. In Korea, the change in the distribution of *emm* genotypes rather than the decrease of antibiotic consumption might have affected the resistance rate. The macrolide resistance was correlated with *emm* genotype. The most resistant genotype was *emm*12.
Conclusion: The erythromycin resistance rate has remained quite low in Korea since 2004. The current situation of the decline of erythromycin resistance in Korea despite increased macrolide usage is curious, because erythromycin resistance of *S. pyogenes* seemed not affected by antibiotic selective pressure.

P1-ST14
Fluoroquinolone resistance in *Streptococcus pneumoniae* from patients with pneumococcal diseases in a medical center in Taiwan

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Background: Resistance to fluoroquinolones (FQ) was associated with clinical failure when treating pneumococcal diseases. We collected clinical isolates of *Streptococcus. pneumoniae* from 2011 to 2014 at Chang Gung Memorial Hospital. Antimicrobial susceptibility and molecular epidemiology of the isolates were studied.
Method: Susceptibility to FQ was examined by disk diffusion method. Levofloxacin ormoxifloxacin non-susceptible *S. pneumoniae* isolates were analyzed by serotyping, multilocus sequence typing, and sequencing of quinolone resistance-determining regions of the *gyrA*, *gyrB*, *parC*, and *parE*.
Result: In these 4 years, 39 FQ non-susceptible *S. pneumoniae* isolates were identified. The rate increased from 1.6% (2 of 127) in 2011, 3.6% (13 of 359) in 2012, and 3.5% (11 of 310) in 2013, to 4.6% (13 of 283) in 2014.The isolates belonged to 13 serotypes and serotype 14 (10 of 39, 25.6%) was the most prevalent. We identified 19 genotypes and the most prevalent was ST876 (9 of 39, 23%); all were serotype 14. Five of the 6 blood isolates were PCV13 serotypes. In 6 (15.4%) isolates from pediatric patients, 4 were acute sinusitis isolates and all belonged to non-PCV13 serotypes. Thirty-two (82%) isolates were susceptible to penicillin (MIC ≤2 μg/mL) but only 7 (17.9%) were susceptible to ceftriaxone (MIC ≤0.5 μg/mL). Alterations in GyrA were found in 38 isolates, mostly in S81 (36 of 39, 92%), and 13(36.1%) of them had another alteration in S114. Alterations in S79 of the ParC (33 of 39, 84.6%) was another frequent alteration. In ParE the most frequent alteration was in D435 (15 of 39, 38.5%). None had *GyrB* alteration.
Conclusion: FQ resistance emerged in either PCV13 or non-PCV13d serotypes. Most of the resistant isolates remained susceptible to penicillin. Increasing trend of FQ resistance in *S. pneumoniae* poses a threat to public health in Taiwan.

P1-ST15
The serotype distribution and antibiotic resistance of *Streptococcus pneumoniae* showed distinct discrepancy between the inpatients' and outpatients' isolates in Beijing Children's Hospital, China

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Background: To compare the serotype distribution and antibiotic resistance of *Streptococcus pneumoniae* (*S. pneumoniae*) isolated from inpatients and outpatients in one hospital.
Methods: All non-invasive isolates from the children under 5 years of age who were admitted to inpatient department from March 2013 to February 2014 were involved. Equal amount of nasopharyngeal isolates from same age group children visiting the outpatient department for upper respiratory infection were randomly choosen for comparisons. The serotype was typed using Quellung reaction with antisera, the antibiotic resistance against 13 antimicrobials were tested using the E-test method or disc diffusion.
Results: 140 non-invasive pneumococcal isolates were collected from inpatient department. The prevailing serotypes were 19F (32.9%), 19A (20.7%), 23F (10.7%), 6A (10.0%), 14 (8.6%) and 15B (6.4%). The coverage rates of 7-valent pneumococcal conjugate vaccine (PCV7), PCV10 and PCV13 were 55.7% (78/140), 56.4% (79/140) and 87.9% (123/140), respectively. 140 *S. pneumoniae* isolates were selected randomly from outpatient department. The frequent serotypes were 19F (13.6%), 23F (12.9%), 6A (10.0%), 6B (10.0%), 19A (7.9%) and 34 (5.0%). The coverage rates of PCV7, PCV10 and PCV13 were respectively 40.7% (57/140), 41.4% (58/140) and 61.4% (86/140). The rates of serotype 19F and 19A in inpatients' group were significant higher than the ones in outpatients' group (P<0.05). The coverage rates of the three vaccines in inpatients' group also exhibited significantly higher level (P<0.05). The non-susceptibility rates against penicillin, amoxicillin-clavulanic acid, imipenem, cefuroxime, cefaclor and trimethoprim-sulfamethoazole in inpatients' group (7.1%, 7.1%, 65.7%, 92.8%, 93.6%, and 85%) were higher than those of the outpatients' group (0.7%, 0.7%, 38.6%, 50%, 53.5%, and 65.7%).
Conclusions: The serotypes 19F and 19A were more frequent in *S. pneumoniae* isolates collected from pediatric inpatients than those from outpatients. The PCVs could cover more in inpatient isolates, which were determined more resistance against antimicrobials, especially the beta-lactams.