

extracted and subjected to the analysis using MALDI-TOF mass spectrometry.

Results: Sensitivity to colistin were determined when the MIC is ≤ 2 $\mu\text{g/ml}$ according to CLSI guideline. Of the two hundred strains collected, twenty were found to have MIC ranging from 4 to 128 $\mu\text{g/ml}$. The RAPD grouped the resistance strains into five groups. The MALDI-TOF MS revealed that the basic structure of *A. baumannii* lipid A is the heptaacylated diphosphoryl lipid A (m/z 1910). The resistance strains exhibited the extra peaks at either m/z 2034, 2071 or 2194, which correspond to the additions of phosphoethanolamine, hexosamine or both to lipid A molecule, respectively. The addition of these residues takes place at the phosphate moieties of lipid A thus potentially cancelling the negative charges and may render *A. baumannii* the resistance to colistin.

Conclusion: The analysis of lipid A from Thai *A. baumannii* colistin resistance strains demonstrates the modification by which the negative charges were eliminated by the addition of small residues and may subsequently lead to the reduction of colistin target.

<http://dx.doi.org/10.1016/j.ijid.2012.05.609>

Type: Poster Presentation

Final Abstract Number: 56.061

Session: Antibiotics

Date: Saturday, June 16, 2012

Time: 12:45-14:15

Room: Poster & Exhibition Area

Molecular epidemiology of *Acinetobacter baumannii* integrated with genomic resistance island

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Background: International clones of multi-drug resistant *Acinetobacter baumannii* integrated with Genomic Resistance Islands (GRI); AbaR1, AbaR2, AbaR3, AbaR5 have been identified. The objective of this study was to identify GRI harboring *A. baumannii* in Thailand and to establish its genetic linkage with international clones.

Methods: Thirty seven non-repetitive clinical isolates of carbapenem resistant *A. baumannii* were studied. Dot blot hybridization was performed to screen for class 1 integron element integrase gene (*IntI1*). Cassette characterization of class 1 integron element, GRI junctional typing, and amplification of class 1 integron borne resistance genes were performed by PCR. Real time multiplex-PCR (RT-MPCR) was performed to amplify integron and non-integron borne genes present in GRIs and resistant plasmid; AbaR1, AbaR2, AbaR3, AbaR5, and plasmid pACICU. Clonal evaluation was carried out by Multi-locus Sequence Typing (MLST) and eBurst analysis.

Results: Four GRI junctional types were identified, type I (n = 24, both 5' and 3' junctions present), type II (n = 3, 5' junction only), type III (n = 7, 3' junction only), and type IV (n = 3, no junction identified). The prevalence of class 1 integron element was 81.08% and this integron element harbored *blaVEB-1* (n = 7), *arr-2* (n = 10), *cmlA* (n = 5), *blaOXA-10* (n = 9), and *blaTEM-1* (n = 15) genes. Cassette organization in *blaVEB-1* containing 5.5 kb class 1 integron element was 5'CS-aadB-blaVEB-1-arr-2-cmlA-aadA1-blaOXA-10-3'CS. RT-MPCR identified a presence of *arsC* (n = 32), *aacA4* (n = 23), *aac3* (n = 15),

tional types had unique STs. The eBurst analysis for the first time identified the presence of pan-European clone I and II and Chinese clone ZS4 in Thailand.

Conclusion: The international clones of *A. baumannii* integrated with variants of GRI were prevalent in Thailand. Monitoring and surveillance of these multi-drug resistance clones is necessary to prevent further spread of these international multi-drug resistant clones in Thailand.

<http://dx.doi.org/10.1016/j.ijid.2012.05.610>

Type: Poster Presentation

Final Abstract Number: 56.062

Session: Antibiotics

Date: Saturday, June 16, 2012

Time: 12:45-14:15

Room: Poster & Exhibition Area

The detection of the NDM-1 and other carbapenemase genes in multidrug resistant Gram negative bacilli (MDRGNB) in a Jamaican hospital

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Background: Resistance of clinically significant bacteria to carbapenem antibiotics, through the production of carbapenemases has become a global problem. This has serious implications as infections caused by these resistant organisms are difficult to manage.

Our aims were to determine if carbapenemases were a mechanism of resistance among MDRGNB isolated at the Microbiology Laboratory, University Hospital of the West Indies (UHWI), Jamaica, to identify specific carbapenemase genes and to determine the relatedness of the strains, so that policies regarding infection control and antibiotic therapy could be better informed.

Methods: At UHWI from May 2009 to March 2011, 105 MDRGNB (1 *Klebsiella pneumoniae*, 3 *Escherichia coli*, 82 *Acinetobacter spp.* and 19 *Pseudomonas spp.*) were identified as potential carbapenemase producers during routine susceptibility testing of clinical isolates. Further phenotypic screening for carbapenemase production was performed using inhibitors and the modified Hodge test. Multiplex PCR for identifying OXA-23, 24, 51, 58 was performed on *Acinetobacter spp.* For the remaining isolates, multiplex PCR was performed to detect NDM, KPC, VIM, GES, IMP and OXA-48 carbapenemase genes. PFGE was used to determine the relatedness of carbapenemase-positive isolates.

Results: Of 4 Enterobacteriaceae tested, phenotypic screening and PCR identified the *Klebsiella pneumoniae* as the first isolate in Jamaica to possess the NDM-1 gene. Of the 82 *Acinetobacter spp.*, all but 1 were positive for blaOXA 51, 12 had blaOXA 24, 2 blaOXA23, and 1 blaOXA58. One *P. aeruginosa* had the GES gene, but without sequencing, its carbapenemase activity is uncertain. *Acinetobacter spp.* and *Pseudomonas spp.* sharing similar genotypes were highly related, suggesting an endemic or outbreak strain.

Conclusion: Carbapenemase production is a cause of resistance amongst MDRGNB in Jamaica, some of which are related. Pheno-

typic testing, while useful for class A and metallo-carbapenemases, was found to be of limited use in its ability to detect the presence of OXA-carbapenemases. Due to the prevalence of certain OXA-carbapenemases in *Acinetobacter spp.*, PCR was better able to detect these genes. The identification of the NDM-1 gene and other carbapenemase genes in Jamaica underscores the need for effective surveillance and infection control measures to identify and prevent spread of MDRGNB's.

<http://dx.doi.org/10.1016/j.ijid.2012.05.611>

Type: Poster Presentation

Final Abstract Number: 56.063

Session: Antibiotics

Date: Saturday, June 16, 2012

Time: 12:45-14:15

Room: Poster & Exhibition Area

High prevalence of D-test positivity in clinical isolates of *Staphylococcus aureus* among Japanese children

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Background: Clindamycin is one of options for treating skin and soft tissue infections caused by *Staphylococcus aureus*. Little is known regarding to the prevalence of D-test positivity of clinical isolates of *Staphylococcus aureus* among Japanese children. Since D-test positivity is associated with treatment failure by Clindamycin, it is important to know the prevalence to select an optimal first-line therapy.

Methods: We retrospectively reviewed the clinical isolates data of *Staphylococcus aureus* between January 2011 and December 2011 at tertiary Children's hospital in Tokyo. Specimens were collected from both inpatient and outpatient. The antibiotic susceptibility and D-test were performed automatically by MicroScan Walk-Away 96 Plus® (Siemens). The panel PC3.1J (Siemens) was used for analysis. D-test was performed according to M100-S19 Clinical Laboratory and Standards Institute (CLSI) 2009. We reviewed the result of susceptibility and D-test of *Staphylococcus aureus* isolates.

Results: The total of 468 *Staphylococcus aureus* was identified in our study. The isolates collected from outpatient and inpatient was 190 and 278, respectively. Methicillin-sensitive *Staphylococcus aureus* (MSSA) and Methicillin-resistance *Staphylococcus aureus* (MRSA) were 367 (79.4%) and 101 (21.6%), respectively. D-test was performed among 127 MSSA isolates and 44 MRSA isolates. Positive D-test rates for MSSA and MRSA were 93.7% (119/127) and 86.4% (38/44), respectively. Positive D-test rate for MRSA isolates from outpatient and inpatient was 84.6% (11/13) and 87.1% (27/31), respectively. Over all Clindamycin resistance including D-test positive strains for MSSA and MRSA was 34.1% (125/367) and 75.2% (76/101), respectively.

Conclusion: We identified high prevalence of D-test positivity both in MSSA and MRSA among Japanese children. Intravenous Vancomycin is usually the treatment of choice at inpatient. Oral Clindamycin is often preferred at outpatient. In our study, MRSA isolates from outpatient demonstrated high D-test positive rate. D-test is not routinely performed at microbiology laboratory at small institutions in Japan. Oral Clindamycin may not be a suitable option for MRSA treatment.

<http://dx.doi.org/10.1016/j.ijid.2012.05.612>

Type: Poster Presentation

Final Abstract Number: 56.064

Session: Antibiotics

Date: Saturday, June 16, 2012

Time: 12:45-14:15

Room: Poster & Exhibition Area

Survey for carbapenemase-producing *Klebsiella pneumoniae* isolated from clinical specimens in Thai hospitals

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Background: Carbapenems, broad spectrum β -lactam antibiotics are the most effective therapy for various serious infections included those caused by *Enterobacteriaceae*- producing extended spectrum β -lactamases (ESBLs). The rapid spread worldwide of carbapenem resistant *Klebsiella pneumoniae* by producing enzymes *K. pneumoniae* carbapenemases (KPCs) limited the effectiveness of carbapenems in the treatment of multidrug-resistant infections. Adequate detection of carbapenemase-producing *Enterobacteriaceae* in routine diagnostic laboratory is essential for clinical management. This study aimed to detect KPC enzyme production in *K. pneumoniae* isolated from clinical specimens in Thai hospitals.

Methods: A total of 351 *K. pneumoniae* isolates from clinical specimens of 4 hospitals in Bangkok and perimeter areas in 2010 were tested for carbapenem-resistance by ertapenem imipenem and meropenem disk diffusion test, based on the CLSI new breakpoint in June 2010, and Modified-Hodge test (MHT). KPC production was confirmed among the carbapenem disk non-susceptible isolates with *blaKPC* gene detection by PCR.

Results: Among 351 *K. pneumoniae* isolates, 18 (5.13%) were ertapenem non-susceptible isolates and 3 isolates (0.85%) produced MHT positive results. All ertapenem non-susceptible isolates were *blaKPC* gene negative, except one isolate. The MHT positive isolate that possessed *blaKPC* gene, was an MDR-ESBL producing *K. pneumoniae* recovered from respiratory tract of an infected patient. It was highly resistant to both ertapenem and imipenem and intermediate resistant to meropenem (MICs of ertapenem, imipenem and meropenem by E-test; 8, 8 and 3 mg/L, respectively).

Conclusion: Results demonstrated the high prevalence of *K. pneumoniae* resistance to carbapenems among isolates from clinical specimens in Thai hospitals. The carbapenems resistant isolates might have other resistant mechanisms than the production of enzyme KPC. These isolates may have impact in clinical management, therefore future clinical study of infections from this resistance organism and development for more effective assay in routine detection of KPC are required. This study implies that implementing guidelines for detecting carbapenem-resistant *Enterobacteriaceae* are necessary for antimicrobial resistance surveillance system in Thailand. Moreover, to contain this resistant organism, an aggressive infection control strategy should early be established.

<http://dx.doi.org/10.1016/j.ijid.2012.05.613>