O017

2-hour Oral Session

Molecular characterization of carbapenem-resistant *Klebsiella spp* in Singapore: OXA-carbapenemase is the main culprit

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Objectives
The incidence of carbapenem-resistant *Enterobacteriaceae* (CRE) in our hospital has been steadily increasing since 2010. Treatment of these CRE infections is challenging, often requiring combination therapy. In our previous studies, we observed that there was much variability in the *in vitro* bactericidal activity of combination antibiotics among these CRE isolates. This could potentially be related to the differences in their underlying resistance mechanisms. The objective of this study was to characterize the molecular resistance mechanism profile of carbapenem-resistant *Klebsiella spp* (CRKS) in our institution.

Methods
100 non-repeat *Klebsiella spp* clinical isolates, which were screened positive for potential carbapenemase production, from a local 1700-bed academic hospital were collected between 2011 - 2014 and characterized. MICs were tested using the reference microdilution method and interpreted in accordance to the 2014 CLSI guidelines. Resistance gene analyses of extended-spectrum beta-lactamases (ESBLs) (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{AmpC}) and carbapenemases (bla_{VIM}, bla_{IMP}, bla_{KPC}, $bla_{OXA-48like}$, bla_{NDM}) were performed using PCR. Changes in expression levels of ompK35 and ompK36 porin genes were detected using RT-PCR. Presence of efflux pumps was tested using efflux inhibitor Phe-Arg β-naphthylamide dihydrochloride (PABN) (50ug/ml).

Results
Out of the 100 isolates, 21 were multi-drug resistant, 72 were extensively-drug resistant, while the rest were pan-drug resistant. The MIC_{50} (range) was ≥32ug/ml (0.12 - ≥32ug/ml); ≥32ug/ml (0.5 - ≥32ug/ml); 4ug/ml (0.25 - ≥32ug/ml); 4ug/ml (0.25 - ≥32ug/ml) for ertapenem, imipenem, meropenem and doripenem respectively. Susceptibility rates of the other antibiotics are as follows: Levofloxacin (23%); aztreonam (3%); piperacillin/tazobactam (2%); cefepime (2%); amikacin (64%). Tigecycline MIC_{50} (range) was 2ug/ml (≤0.25 - ≥64ug/ml), while that of polymyxin B was 1ug/ml (0.5 - ≥32ug/ml) Carbapenemases were responsible for mediating resistance in 86 isolates. $bla_{OXA-48/181}$ genes (47) were most commonly detected, followed by bla_{KPC} (27), bla_{NDM} (27) and bla_{IMP} (3). Out of these, 17 were $bla_{OXA-48like}$ and bla_{NDM} co-producers. ESBLs were also highly prevalent (bla_{TEM} and/or bla_{SHV}: 99 isolates; bla_{CTX-M}: 70 isolates; bla_{AmpC}: 14 isolates). Decreased expression of porin genes was detected in 30 isolates [ompK35 (3); ompK36 (19); both (8)]. Addition of PABN resulted in a 8-fold decrease in carbapenem MICs only in one isolate. Carbapenem resistance was mediated by both carbapenemase prodcution and porin loss±ESBL production in 24 isolates. It appears that there is no single dominant mechanism type responsible for mediating high-level carbapenem resistance (MIC ≥ 16ug/ml).

Conclusions
There was a diversity of mechanisms mediating carbapenem resistance in CRKS in our institution. OXA-48-type carbapenemase was the dominant mechanism in our CRKS population. The variety of underlying resistance mechanisms presents a complexity in the selection of appropriate empiric therapy for such infections in the local setting, suggesting the importance strain-specific combination testing to guide therapy.