

Characterization of Carbapenem Resistant Metallo-beta-lactamase Producing *Klebsiella pneumoniae* from Pranangklaao Hospital

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Background: Carbapenem resistant *Klebsiella pneumoniae* has been increasing in many countries due to clonal expansion. We have isolated a carbapenem resistant *Klebsiella pneumoniae* from sputum specimen obtained from one female patient. This was the first carbapenem resistant *K. pneumoniae* isolate from this hospital to be studied and characterized for its mechanism used for carbapenem resistance.

Methods: The sputum specimen has been worked up for the causative agent of pneumonia in one female patient. The species identification was done by the standard conventional methods used in the clinical microbiology laboratory. The antibiotic susceptibility test using the Kirby-Bauer disk diffusion method has been performed to determine its carbapenem resistance. The modified Hodge test was performed to determine whether carbapenem resistance was caused by the production of carbapenem-hydrolyzing enzyme. Double disk diffusion technique for detection of metallo-beta-lactamase production has been tested. Specific polymerase chain reactions for *bla*_{KPC}, *bla*_{OXA}, *bla*_{VIM}, and *bla*_{IMP} detections have been used for

determination of the family of beta-lactamase enzyme produced by this resistant isolate. Specific primers for 5' to 3'-conserved segment were used to amplify resistant gene cassette carried by this isolate. PCR product was used for gene cloning and DNA sequencing experiment to determine the type of metallo-beta-lactamase.

Results: Carbapenem resistant *K. pneumoniae* showed positive result on the modified Hodge test, which prompted us to search for the production of *bla*_{KPC}, however it was not identified in this isolate. Upon testing on the production of metallo-beta-lactamase, there was positive amplified DNA fragment from PCR primers specific for *bla*_{IMP} with 571 bp band. The PCR product from 5' to 3'-conserved segment gave 1,857 bp band of resistant gene cassette, and the DNA has been cloned into the plasmid pSC-A-Amp/Kan (StrataGene[®], USA), and recombinant plasmid has been sent for DNA sequencing.

Conclusion: There was a carbapenem resistant *K. pneumoniae* capable of producing carbapenem-hydrolyzing enzyme in Pranangklaao Hospital. The

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screening test using disk diffusion method and PCR amplification confirmed the presence of metallo-beta-lactamase of the *bla*_{IMP} family. Furthermore, from restriction endonuclease analysis by using 1,857 bp PCR product revealed specific digested DNA fragments

similar to the previous report of *Pseudomonas aeruginosa* carrying the *bla*_{IMP-15} class B metallo-beta-lactamase gene. The finding indicated the emergence of highly resistant enteric bacteria via spread of resistance genes.