UNIVERSITI TEKNOLOGI MARA

MOLECULAR STUDIES OF ANTIBIOTIC RESISTANCE AMONG THE CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* ISOLATED IN SELAYANG HOSPITAL, MALAYSIA

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of the requirements for the degree of
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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This topic has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research

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ABSTRACT

This study was carried out to determine the molecular analysis of antibiotic resistance among the clinical isolates of *Pseudomonas aeruginosa.* For this study, 54 *P. aeruginosa* isolates were obtained from blood, skin, pus, respiratory, eyes, urine and sputum of both paediatric and adult patients. Antibiotic sensitivity analysis was carried out using showed Quinolone (Ciprofloxacin) to be the most active antimicrobial agents with 83.34 % susceptibility followed by imipenem (81.49%), aminoglycosides (amikacin,74.08% and gentamicin, 72.23%) and the beta- lactams (cefepime 62.97%, ceftazidime (15%) and amikacine (14%). Among all the *P. aeruginosa* isolates, 29% of the strains were resistant to one antibiotic, 20% strains were resistant to two antibiotics and 51% were multidrug resistance. All the 54 strains of *P. aeruginosa* were subjected to MIC determination for ceftazidime and piperacillin by E-test. Piperacillin and ceftazidime showed the maximum number of resistance against *P. aeruginosa* 50% and 29.63% respectively. Among all the *P. aeruginosa* isolates, 12.97% were ESBL positive in phenotypic test whereas the 11.12% were found positive using E-test. But none of the isolates were found to produce MBLs. All these isolates were examined for the production of plasmid and were further analysed. Supercoil DNA marker was used to determine the plasmid sizes. It was used in electrophoresis gel each time along with the plasmids as molecular weight marker. Plasmid profiling analysis shown that, DNA marker was used to estimate the plasmid size and was used as molecular weight markers in each gel running. Plasmids were detected in 10 isolates with the POR of (18.51%) by using Close and Rodriguez with modification (1982) and QIAprep Spin Kit. The overall sizes of the plasmid DNA range from the lowest 1.8 kb to the highest 14 kb. These 10 isolates appear to harbor 1 or more plasmids with the maximum of 4 plasmids. Various plasmid profiles were observed in the isolates. The plasmid analyses revealed that detectable plasmids in 10 (29.42%) out of the 34 selected multi-drug resistant *P. aeruginosa* isolates. Twenty four of the isolates possessed no plasmids, 3 isolates possessed single sized plasmids (3400bp- 4600bp) while 3 isolates had four plasmids with sizes from (1800bp- 5800bp). Four isolates which had 2 plasmids size of (220bp-14000bp) respectively. PCR were used to detect the ESBL genes. In this study, we examined the molecular type of *blaSHV, SHV, TEM* and *CTX-M* derived ESBL variants produced by clinical *P. aeruginosa* isolates. We identified the strains carrying *blaSHV* and *SHV* genes by PCR test and confirmed the results by nucleotide sequencing. Polymerase chain reaction used in this study proved to be rapid reproducible and specific. We have found that 6 *P. aeruginosa* isolates carried *blaSHV* gene whereas 2 *P. aeruginosa* isolates carried SHV gene. Polymerase chain reaction produces a fragment of 200bp of *blaSHV* gene and 475bp of *SHV* gene respectively. ESBL positive isolates were found to harbour 1 or more plasmids. But *TEM* and *CTX-M* genes were not found in all the ESBL positive strains. The *SHV* & *blaSHV* genes were amplified by PCR. Approximately, 10-12 PCR reaction was carried out to have adequate amount of gene for sequencing and combined together to pool and purified by PCR purification kit. The purified product for sequencing service was sent to Medigene Sdn Bhd Selangor, Malaysia. The sequencing result was analysed online using NCBI and BLAST software.

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