

Molecular Characterization of Metallo-β-Lactamase Genes and Integrations in *Acinetobacter baumannii* Isolated from Taiwan

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ABSTRACT

Acinetobacter baumannii is a common opportunistic pathogen, which constantly brings the prevalence of infection among hospitals. Clinically, the antibiotics were used so frequently that multidrug-resistant *A. baumannii* has emerged quickly and caused the difficulties in therapies. Since the increasing rate of *A. baumannii* infection from 0% in 1998 to 6.5% in 2000 at National Taiwan University Hospital, the multidrug-resistant *A. baumannii* isolates have spread rapidly and caused the seriousness of nosocomial infection. Therefore, the objective of this research is to understand the genes related to resistance to imipenem and the mode of its spreading in multidrug-resistant *A. baumannii* isolates. In clinical therapy, imipenem (a β-lactam type antibiotic) is the major drug for those who were infected with *A. baumannii*. Thus this research will study the resistance to imipenem. One hundred and eighty-eight isolates of *A. baumannii* used in this research were isolated from hospitalized patients in Chia-Yi and Lin-Kou Chang Gung Memorial Hospitals. After antimicrobial susceptibility testing, two imipenem-resistant isolates from Chia-Yi (P-78 and P-210), plus six imipenem-resistant isolates from Lin-Kou (AB-394, AB-1756, AB-1757, AB-1758, AB-1759 and AB-1760) were selected. The analysis and comparison study of their drug resistance related genes and possible spreading mode were also performed. In addition to that, two isolates (P-21 and P-23) which were susceptible to imipenem but resistant to other tested antibiotics, and one isolate (P-2) which was susceptible to all tested antibiotics were also included. Primers specific for metallo-β-lactamase genes, blaIMP, blaVIM, and cfiA, were designed for PCR amplification and sequence analysis. Only isolates AB-394 and P-78 were identified possessing resistance gene blaIMP-1. On the other hand, none of isolates P-210, AB-1756, AB-1757, AB-1758, AB-1759 or AB-1760 were proven to contain blaIMP, blaVIM or cfiA. Furthermore, plasmid DNA of these isolates were analyzed by the method of Kado and Liu and transferred to nylon membrane, and PCR product of blaIMP-1 was used as probe to perform Southern hybridization. The results showed that blaIMP-1 was located on the large plasmid (>100 kb) in isolate AB-394 while the other was on the plasmid of around 95 kb in isolate P-78. Given that isolates P-210, AB-1756, AB-1757, AB-1758, AB-1759 and AB-1760 did not harbor any plasmid, the drug resistance genes other than metallo-β-lactamase genes of these isolates differed from that described above, may be most likely located on the chromosome. However, the identification of this type of drug resistance mechanism in these isolates will need further research. Meanwhile, we further analyzed the structure of integron which may carry drug resistance gene. Based on the nucleotide sequences of the 5' -integron I conserved region, PCR primers were designed and used to identify that all of 10 isolates P-21, P-23, P-78, P-210, AB-394, AB-1756, AB-1757, AB-1758, AB-1759 and AB-1760 appeared to have integron I sequence. After purification and sequence analysis of PCR products, we found isolates AB-394 and P-78 have the same integron I structure and contained several resistance genes including blaIMP-1, which was absent in the integron I structure of the isolates of P-210, AB-1756, AB-1757, AB-1758, AB-1759 and AB-1760. Then, the phylogenetic differences among these *A. baumannii* isolates were analyzed by pulsed-field gel electrophoresis (PFGE). The results showed that the recently isolated multidrug-resistant isolates (AB-1758, AB-1759, AB-1760 and P-23) very likely belong to the same clone, while isolates P-210 and P-21 are very closely related. Up to date, Taiwan is still at the initial stage of probing into the imipenem resistance mechanisms, including integrons, from clinical isolates of *A. baumannii*. This research will help to the understanding of epidemiology and the control of infection caused by *A. baumannii*.

Keywords : *Acinetobacter baumannii* , drug resistance gene , integron , PFGE

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