Application of insertion sequence on genotyping and antimicrobial resistance gene identification

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ABSTRACT

Acinetobacter baumannii is an opportunistic pathogen, usually causing nosocomial infections in immunocompromised and elderly petients. Current antibiotics commonly used against A. baumannii are imipenem. In recent years, the imipenem resistance of A. baumannii is dramatically increased, due to the presence of ISAba1 at the upstream of a beta-lactamase gene, blaOXA-23 or blaOXA-51. ISAba1 inserted into the upstream of resistance genes can not only enhance gene expression, but also form a transposon, and therefore allow the nondrug-resistant bacteria to become drug-resistant, and facilitate the spread of drug resistance genes. In this study, sixty-two clinical A. baumannii isolates were collected for genotyping during from 2009 to 2010 from two hospitals in northern Taiwan, including Chang Gung Memorial Hospital and St. Paul ’ s Hospital. First, 61 of 62 isolates were divided into 41 genotypes by pulse field gel electrophoresis (PFGE); 13 genotypes were classified by PCR typing. This typing was developed to alternatively group A. baumannii isolates among the diverse PFGE genotypes in this study. We used two individual sets of nested PCR primers, where one side of two individual primer sets was chose according to a known gene sequence, i.e. a specific insertion sequence ISAba1. However, the other side could be any unknown DNA sequence, which may be targeted by a semi-random primer with a 14-base random sequence at the 3 ’ end of primer and a 26-base multi-cloning-sites sequence at the 5 ’ end, where this 26-base sequence may be used as a primer for the second round of PCR. When combining PFGE and PCR genotypes together with multi-drug resistance gene patterns, we found a major group of type I isolates that containing multiple drug-resistant genes or transposon-related sequences, including ISAba1-blaOXA-51-like (Tn6080-like), ISAba1-blaOXA-23-ISAba1 (Tn2006), and ISAba1- blaOXA-23 (Tn2008). In contrast, type II or XIII isolates were in a minority without any common drug-resistant gene detected. We also used the same PCR method to sequence an unknown resistance gene that was flanked by a specific insertion sequence ISAba1 for A. baumannii or ISEcp1 for Klesiella pneumoniae and Escherichia coli isolates. The sizes of any PCR-amplified fragments selected for DNA sequencing and NCBI database annotation were larger than 550 bp long. Only five of twelve A. baumannii resistance genes detected beside ISAba1 were in consistent with the previously verified resistance genes. However, when detecting the flanking sequence of ISEcp1 in five of each extended-spectrum beta-bactamases-containing K. pneumoniae and E. coli isolates, seven of them promisingly exhibited the same results with the known data. Our study may offer an information for A.baumannii genotyping and an alternative approach to search drug resistance genes, and the resulting data may contribute to clinical antibiotic treatment.

Keywords : ISAba1、PCR-typing、OXA、Acinetobacter baumannii、PCR-typing

Table of Contents

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