Original Article
Investigation on the genomic diversity of OXA from isolated Acinetobacter baumannii

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Received January 12, 2015; Accepted February 25, 2015; Epub March 15, 2015; Published March 30, 2015

Abstract: We distinguished the four alleles of OXA subgroups from 138 strains of Acinetobacter baumannii using Polymerase Chain Reaction, and investigated distributions of OXA subgroups in clinical isolated strains. A total of 170 Acinetobacter baumannii were isolated from Shenzhen Longgang Central Hospital between 2010 and 2013. Amplification of OXA genes, blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58, were performed by multiplex PCR. Multiplex PCR results showed, out of the 96 strains of Acinetobacter baumannii, 50 (52.08%) strains were positive for only blaOXA51 gene, and 46 (47.92%) showed positive for both blaOXA51 and blaOXA58 genes. Among 96 strains of Acinetobacter baumannii, 48 strains were resistant to carbapenems, and 48 strains were sensitivity to carbapenems. blaOXA51 and blaOXA58 showed resistant or sensitivity to carbapenems. In conclusion, we found that blaOXA-51 and blaOXA-5 were the main mechanisms of resistant or sensitivity to carbapenems.

Keywords: Genomic diversity, OXA, Acinetobacter baumannii

Introduction

Acinetobacter baumannii is one kind of aerobic non-motive gram-negative coccobacillus. Polymorphic bacterial pathogen of Acinetobacter baumannii is easily spread between patients, which can persist in the environment for several days [1]. It is reported that Acinetobacter baumannii is a most common pathogenic bacteria isolated from hospitalized patients with pneumonia, which have an important role in nosocomial infections [2, 3]. It is well known that Acinetobacter is a common nosocomial pathogen, and is widely found in intensive care units (ICUs) and can cause severe infections. Acinetobacter baumannii usually show multi-drug resistant to many drugs, such as third generation cephalosporins, aminoglycosides and fluoroquinolone [4]. It is reported that the most common mechanism of drug resistance is the correlation between hydrolyzingβ-lactamases of metallo-β-lactamases (Ambler class B) and oxacillinases (Ambler class D). Widely use of antimicrobial chemotherapy can have an important role in the appearance of carbapenem-hydrolyzing class D β-lactamases (CHDLs), which are widely identified in Acinetobacter baumannii. There are four subgroups of acquired CHDLs in Acinetobacter baumannii, including blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58. Therefore, the aim of our study was to distinguish the four alleles of OXA subgroups from 138 strains of Acinetobacter baumannii using Polymerase Chain Reaction, and investigate distributions of OXA subgroups in clinical isolated strains.

Methods and materials

A total of 170 Acinetobacter baumannii were isolated from Shenzhen Longgang Central Hospital between 2010 and 2013. The strains were identified as Acinetobacter baumannii by multiple Polymerase Chain Reaction (PCR) test. Two amplification bands were determined as Acinetobacter baumannii. Finally, 138 strains of Acinetobacter baumannii were identified, and we selected 96 strains to detect the distributions of OXA gene , in which 48 strains were sensitivity to carbapenems and 48 strains were resistant to carbapenems.
OXA genomic diversity in Acinetobacter baumannii

<table>
<thead>
<tr>
<th>PCR-Positive for Genes</th>
<th>Resistant to Carbapenems (%)</th>
<th>Sensitivity to Carbapenems (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA23</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>OXA24</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>OXA51</td>
<td>9 (9.38)</td>
<td>41 (42.71)</td>
</tr>
<tr>
<td>OXA58</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>OXA51 + OXA58</td>
<td>38 (39.58)</td>
<td>6 (6.25)</td>
</tr>
<tr>
<td>OXA51 + OXA23</td>
<td>1 (1.42)</td>
<td>1 (1.42)</td>
</tr>
</tbody>
</table>

PCR amplification

Amplification of OXA genes, blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58, were performed by PCR. The PCR analysis were performed using the primers as follows: for blaOXA-23, the primer sequences were 5'-GATCGGATTGGAGAACCAGA-3' (forwards) and 5'-ATTCTTGACCCGATTCCATTAA-3' (reverse); for blaOXA-24, 5'-GGTTAGTTGGCCCCCTTAAA-3' (forwards) and 5'-AGTTGAGCGAAAAGGGGATT-3' (reverse); for blaOXA-51, 5'-TAATGCYTTGATCGGCGCTTG-3' (forwards) and 5'-TGATTGCACCTCATCTTGG-3' (reverse); for blaOXA-58, 5'-AAGTATTGGGGCTTGTGCTG-3' (forwards) and 5'-CCCCTCTGCGCTCTACATAC-3' (reverse).

Culturing of strains

The isolated strains of Acinetobacter baumannii were stored at -70°C until use. The Acinetobacter baumannii was recovered by Mueller-Hinton agars, and cultured in constant temperature incubator at 37°C. The typical single colony was cultured in MHB at 37°C, and put into constant temperature concussion incubator for 12-16 hours with 180 rpm speed.

Discussion

It is well known that multi-drug resistant Acinetobacter baumannii has emerged as a troublesome nosocomial pathogen worldwide. Acquired OXA carbapenemases was firstly reported in 1993, and the emergence and spread of OXA enzymes have been reported worldwide. It is known that the most common mechanism of drug resistance of Acinetobacter baumannii is the correlation between hydrolyzing β-lactamases of metallo-β-lactamases (Ambler class B) and oxacillinases (Ambler class D), and there are four subgroups of acquired CHDLs in Acinetobacter baumannii, including blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58. Previous studies reported that blaOXA-23 and blaOXA-51 are the most common detected genes in Acinetobacter [5]. blaOXA-24 in Acinetobacter baumannii was reported to be detected in Spain and Iran [6, 7]. blaOXA-58 was reported to be sequential outbreaks in a Saudia Arabia [8].

In our study, most strains were blaOXA-51 and blaOXA-51+blaOXA-58, which is globally scattered among Acinetobacter baumannii isolates, and we found that blaOXA-51 and blaOXA-58...
were the main mechanisms of resistant or sensitivity to carbapenems. The results of our study were similar with previous ones [6, 9, 10]. Bali et al. reported that blaOXA-23 and blaOXA-51 were the major pathogen for carbapenem-resistant Acinetobacter [6]. Mohajeri et al. reported that the blaOXA-51like and blaOXA-23like were the predominant mechanisms of resistance to imipenem in Acinetobacter baumannii [9]. Alvargonzalez et al. reported that all isolates of multidrug-resistant Acinetobacter baumannii contained the blaOXA-51like and OXA-23 genes [10]. However, previous Chinese studies reported the inconsistent results with ours [11-13]. One study reported that only blaOXA-23 and blaOXA-51 were amplified in 96.7% of the Scinetobacter baumannii strains, but the gene blaOXA-24 and blaOXA-58 were not amplified [11]. Ji et al. reported that the blaOXA-24 and blaOXA-58 gene have emerged as potential threats of hospital outbreaks of multidrug-resistant Acinetobacter baumannii [13]. The discrepancies between studies may be due to differences in samples selection and gene variations in different ethnicities as well as sample size. Therefore, further studies are greatly needed to confirm our finding.

In conclusion, we found that blaOXA-51 and blaOXA-58 were the main mechanisms of resistant or sensitivity to carbapenems in our hospital from 2010-2013. With increase in drug resistance in Acinetobacter, resistance surveillance has become increasingly important. Hence both the phenotypic and genotypic methods are important to detect the carbapenem resistance in Acinetobacter and techniques like Multiplex PCR would help to monitor the emergence and spread of carbapenem resistant Acinetobacter.

Disclosure of conflict of interest
None.

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References


