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Original Article

Investigation on the genomic diversity of OXA from isolated *Acinetobacter baumannii*

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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, *bla*OXA-23, *bla*OXA-24, *bla*OXA-51 and *bla*OXA-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 *bla*OXA51 gene, and 46 (47.92%) showed positive for both *bla*OXA51 and *bla*OXA58 genes. Among 96 strains of *Acinetobacter baumannii*, 48 strains were resistant to carbapenems, and 48 strains were sensitivity to carbapenems. *bla*OXA51 and *bla*OXA58 showed resistant or sensitivity to carbapenems. In conclusion, we found that *bla*OXA-51 and *bla*OXA-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-motile 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 multidrug 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 *bla*OXA-23, *bla*OXA-24, *bla*OXA-51 and *bla*OXA-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.

Table 1. Resistant or sensitivity to carbapenems of *bla*OXA genes in *Acinetobacter baumannii*

PCR-positive for genes	Resistant to carbapenems (%)	Sensitivity to carbapenems (%)
OXA23	0 (0)	0 (0)
OXA24	0 (0)	0 (0)
OXA51	9 (9.38)	41 (42.71)
OXA58	0 (0)	0 (0)
OXA51+ OXA58	38 (39.58)	6 (6.25)
OXA51+ OXA23	1 (1.42)	1 (1.42)

PCR amplification

Amplification of OXA genes, *bla*OXA-23, *bla*OXA-24, blaOXA-51 and blaOXA-58, were performed by PCR. The PCR analysis were performed using the primers as follows: for *bla*OXA-23, the primer sequences were 5'-GATCGGATTGGAGAACCAGA-3' (forwards) and 5'-ATTTCTGACCGCATTTCCAT-3' (reverse); for *bla*OXA-24, 5'-GGTTAGTTGGCCCCCTTAAA-3' (forwards) and 5'-AGTTGAGCGAAAAGGGGATT-3' (reverse); for *bla*OXA-51, 5'-TAATGCTTTGATCGGCCTTG-3' (forwards) and 5'-TGGATTGCACTTCATCTTGG-3' (reverse); for *bla*OXA-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-Hinto 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.

The genomic DNA extraction wad performed by pure fast bacterial genomic DNA purification kit from all *Acinetobacter baumannii*. Each PCR reaction mix comprised 50 ng genomic DNA, 200 μM dNTP, 2.5 U Taq DNA polymerase (Promega, Madison, WI, USA), and 200 μM primers, in a total volume of 20 μL. The PCR reaction were preliminary denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 25 s, and annealing at 53°C for 40 s, with a final extension at 72°C for 6 min. The PCR products were verified by 1.0% agarose gel electrophoresis and visualized using ethidium bromide staining and UV light. Reproducibility was verified by repeating analysis of a randomly chosen subgroup of 10% of the subjects.

Statistical analysis

Statistical analyses were conducted using the SPSS® statistical package, version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Categorical variables were expressed by frequency and percentage. All *P*-values were two sided, and a *P*-value < 0.05 was considered statistically significant.

Results

Among 138 strains of *Acinetobacter baumannii*, 96 strains were identified and genotyped for the distributions of OXA gene (**Table 1**). Multiplex PCR results showed, out of the 96 strains of *Acinetobacter baumannii*, 50 (52.08%) strains were positive for only *bla*OXA51 gene, and 46 (47.92%) showed positive for both *bla*OXA51 and *bla*OXA58 genes (**Figure 1**). Two strains (2.08%) showed positive for both *bla*OXA51 and *bla*OXA23 genes. However, we did not find strains with positive *bla*OXA-23 and *bla*OXA24.

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 *bla*OXA-23, *bla*OXA-24, *bla*OXA-51 and *bla*OXA-58. Previous studies reported that *bla*OXA-23 and *bla*OXA-51 are the most common detected genes in *Acinetobacter* [5]. *bla*OXA-24 in *Acinetobacter baumannii* was reported to be detected in Spain and Iran [6, 7]. *bla*OXA-58 was reported to be sequential outbreaks in a Saudia Arabia [8].

In our study, most strains were *bla*OXA-51 and *bla*OXA-51+*bla*OXA-58, which is globally scattered among *Acinetobacter baumannii* isolates, and we found that *bla*OXA-51 and *bla*OXA-58

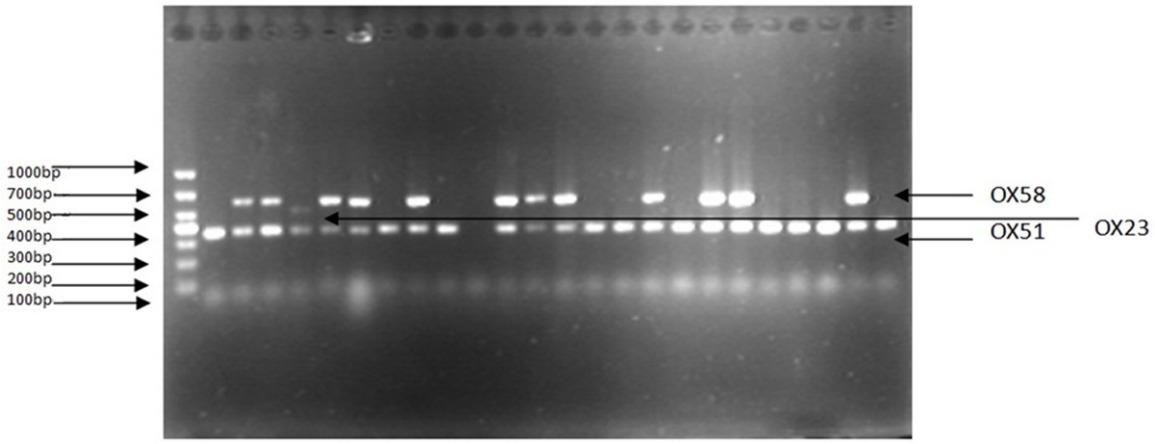

Figure 1. Detection of genes encoding OXA51 and OXA58 genes. Moreover, we detected whether the *Acinetobacter baumannii* showed resistant or sensitivity to carbapenems (**Table 1**). Among 96 strains of *Acinetobacter baumannii*, 48 strains were resistant to carbapenems, and 48 strains were sensitivity to carbapenems. Among 42 strains with positive *bla*OXA51, we found that 9 strains (9.38%) showed resistant to carbapenems, and 41 (42.71%) showed sensitivity to carbapenems. Of 44 strains with both *bla*OXA51 and *bla*OXA58, 38 strains (39.58%) showed resistant to carbapenems, and 6 (6.25%) presented sensitivity to carbapenems. Of two strains with both *bla*OXA51 and *bla*OXA23, one strain showed resistant to carbapenems (1.04%), and another showed sensitivity to carbapenems (1.04%).

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 *bla*OXA-23 and *bla*-OXA-51 were the major pathogen for carbapenem-resistant Acinetobacter [6]. Mohajeri et al. reported that the bla *bla*OXA-51-like and bla *bla*OXA-23 like 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 *bla*OXA-51-like and OXA-23 genes [10].

However, previous Chinese studies reported the inconsistent results with ours [11-13]. One study reported that only *bla*OXA-23 and *bla*-OXA-51 were amplified in 96.7% of the *Scinetobacter baumannii* strains, but the gene *bla*OXA-24 and *bla*OXA-58 were not amplified [11]. Ji et al. reported that the *bla*OXA-24 and *bla*OXA-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 *bla*OXA-51 and *bla*OXA-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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