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Original Article

Drug-resistant gene of *blaOXA-23*, *blaOXA-24*, *blaOXA-51* and *blaOXA-58* in *Acinetobacter baumannii*

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Abstract: We distinguished the four alleles of OXA subgroups from 339 strains of *Acinetobacter baumannii* using Polymerase Chain Reaction, and investigated distributions of OXA subgroups in clinical isolated strains. A total of 196 *Acinetobacter baumannii* were isolated from the Central Hospital of Zhumadian between 2010 and 2014. Amplification of OXA genes, *bla*OXA-23, *bla*OXA-24, *bla*OXA-51 and *bla*OXA-58, were performed by PCR. Patients with Acinetobacter baumannii were selected from ICU, pneumology, emergency and cerebral surgery, accounting for 33.67%, 17.86%, 16.33% and 32.14%, respectively. Most strains showed resistance to different classes of agents, especially in ceftazidime, piperacillin, cefepime, nitrofurantoin and ertapenem. Multiplex PCR results showed, out of the 339 isolated strains, 164 (48.38%) were *bla*OXA-51, 157 (46.31%) were *bla*OXA-23, 18 (5.31%) were *bla*-OXA-58, and no strain for *bla*OXA-24. 143 (47.67%) strains of *bla*OXA-51, 143 (47.67%) strains of *bla*OXA-23, and 14 (4.66%) strains of *bla*OXA-58 showed multidrug-resistant. In conclusion, our study found that OXA-51 and OXA-23 were the main mechanisms of resistant or sensitivity to carbapenems.

Keywords: Genomic diversity, OXA, *Acinetobacter baumannii*

Introduction

Acinetobacter baumannii is an aerobic non-motile gram-negative coccobacillus, and it has become an important hospital-acuired pathogen worldwide, such as pneumonia, septicemia and urinary tract as well as wound infections. Polymorphic bacterial pathogen of *Acinetobacter baumannii* is frequently involved in outbreaks, which can persist in the environment for several days [1]. Previous studies reported that *Acinetobacter baumannii* is a most common pathogenic bacteria isolated from hospitalized patients with pneumonia [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. However, *Acinetobacter baumannii* usually found to have multidrug resistant to many drugs, such as third generation cephalosporins, aminoglycosides and fluoroquinolone [4].

It is reported that multidrug-resistant *Acinetobacter baumannii* strains are gained their antibiotic-resistant genes through class 1 integrons that carry single or multiple gene cassettes [5]. Integrons are genetic elements to encode antibiotic resistance and integrate or mobilize their inherent gene cassettes [6]. Aminoglycoside resistance genes could influence the enzymatic inactivation of aminoglycoside antibiotics, including acetyltransferase, phosphotransferase and adenylyltransferase. Carbapenemase genes are important part of class I integrins, and they could cause carbapenem resistance, such as bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SIM}, or $bla_{OXA\text{-}like}$ [7, 8]. Carbapenemase production is the most well-described resistance mechanism to carbapenems [9]. The mechanisms of drug resistance are usually reported to be correlated with hydrolyzing β-lactamases of metallo-β-lactamases (Ambler class B) and oxacillinases (Ambler class D). Four subgroups of acquired CHDLs in *Acinetobacter baumannii*, including *bla*OXA-23, *bla*OXA-24, *bla*OXA-51 and *bla*OXA-58. In our study, we collected 196 strains of *Acinetobacter baumannii* to detect drug-resistant genes of OXA subgroups by Polymerase Chain Reaction (PCR) analysis, and conduct homology analysis.

Table 1. Characteristics of included patients

Variables	Number N = 196	%
Age		
< 60	38	19.39
≥ 60	158	80.61
Gender		
Male	115	58.67
Female	81	41.33
Location of *acinetobacter baumannii*		
ICU	66	33.67
Emergency	35	17.86
Cerebral surgery	32	16.33
Pneumology	63	32.14

Methods and materials

A total of 339 strains of *Acinetobacter baumannii* were isolated from 196 patients in the Central Hospital of Zhumadian between 2010 and 2014. All the isolated strains were identified as *Acinetobacter baumannii* using multiple Polymerase Chain Reaction (PCR) test.

Antibiotic susceptibility testing

General antimicrobial susceptibilities for *A. baumannii* identification were performed using Vitek 2 Compact system (bioMérieux, Inc., Marcy-l'Etoile, France). Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), and Staphylococcus aureus (ATCC 29213) were taken as quality control strains. Susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

PCR amplification

Two amplification bands were determined as *Acinetobacter baumannii*. Amplification of OXA genes, *bla*OXA-23, *bla*OXA-24, *bla*OXA-51 and *bla*OXA-58, were performed by Taq PCR Master Mix (Shanghai Lifefeng Biotech Co., Ltd, Shanghai, China). 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); The primers for *bla*OXA-24 were 5'-TTCCCCTAACATGAATTTGT-3' (forwards) and 5'-GTACTAATCAAAGTTGTGAA-3' (reverse); The primers for for *bla*OXA-51 were 5'-TAATGCTTTGATCGGCCTTG-3' (forwards) and 5'-TGGATTGCACTTCATCTTGG-3' (reverse); The primers for *bla*OXA-58 were 5'-TGGCACGCATTTAGACCG-3' (forwards) and 5'-AAACCCACATACCAACCC-3' (reverse). The amplicon sizes for *bla*OXA-23, *bla*OXA-24, *bla*OXA-51 and *bla*OXA-58 were 501 bp, 1024 bp, 353 bp and 507 bp.

Table 2. Antibiotics resistance of multidrug-resistant *Acinetobacter baumannii* isolates

Antibiotics	Patients with drug resistant strains	
	Number	%
Ampicillin	162	82.65
Piperacillin	180	91.84
Cefotetan	146	74.49
Cefazolin	154	78.57
Ceftriaxone	143	72.96
Ceftazidime	183	93.37
Cefepime	178	90.82
Cefoperazone	42	21.43
Imipenem	147	75.00
Aztreonam	162	82.65
Amikacin	102	52.04
Gentamicin	129	65.82
Tobramycin	114	58.16
Levofloxacin	128	65.31
Ciprofloxacin	141	71.94
Nitrofurantoin	196	100.00
Ertapenem	196	100.00
Meropenem	150	76.53

Culturing of strains

The isolated strains of *Acinetobacter baumannii* were stored at -70°C until use. The genomic DNA of *Acinetobacter baumannii* was extracted using TIANamp Bacteria DNA Kit (Tiangen, Beijing, China). PCR was performed using Taq PCR Master Mix (TaKaRa Bio, Dalian, China). Each PCR reaction mix was conducted in a 50 μL reaction solution, which contained 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. OXA-51 and 16SrRNA was used as the internal control. The cycling conditions were performed with a 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

Table 3. The PCR results of drug resistant genes

Genes	Total	%	Multidrug-resistant N = 339	%	Non-multidrug resistant N = 39	%
*bla*OXA-51	164	48.38	143	47.67	21	53.85
*bla*OXA-23	157	46.31	143	47.67	14	35.90
*bla*OXA-24	0	0.00	0	0.00	0	0.00
*bla*OXA-58	18	5.31	14	4.66	4	10.26

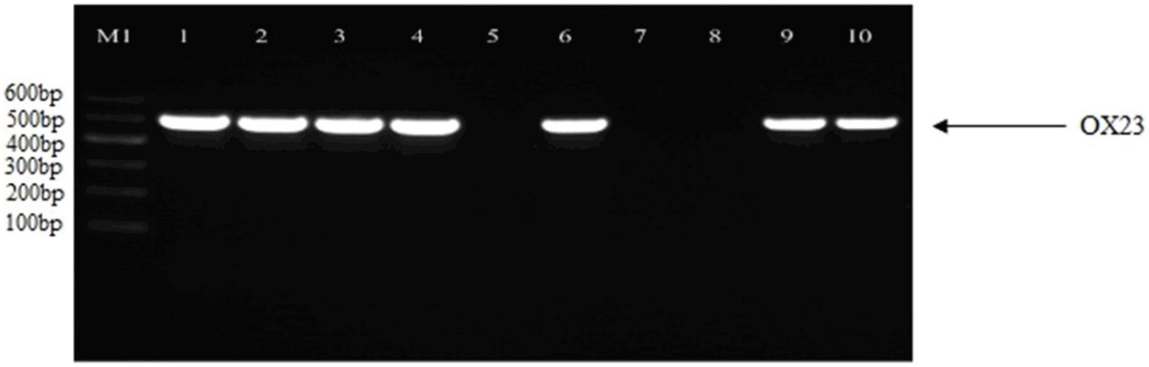

Figure 1. Detection of genes encoding *bla*OXA-23 gene PCR products. Moreover, we detected whether the *Acinetobacter baumannii* showed multidrug-resistant (**Table 3**). Of 339 isolated strains, 164 (48.38%) were *bla*OXA-51, 157 (46.31%) were *bla*OXA-23, 18 (5.31%) were *bla*OXA-58, and no strain for *bla*OXA-24. 143 (47.67%) strains of *bla*OXA-51, 143 (47.67%) strains of *bla*OXA-23, and 14 (4.66%) strains of *bla*OXA-58 showed multidrug-resistant.

bromide staining and UV light. Samples were coded for case-control status, and at least 10% of the samples were randomly selected and subjected to repeat analysis as quality control for verification of genotyping procedures. Two researchers independently reviewed all genotyping results.

Statistical analysis

Statistical analyses were conducted using the SPSS® statistical package, version 16.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Categorical variables were expressed by frequency and percentage, and differences between categorical variables were compared by χ^2 test or Fisher's exact test. All *P*-values were two sided, and a *P*-values less than 0.05 are considered statistically significant.

Results

The characteristics of patients infected with Acinetobacter baumannii

The 349 strains of *Acinetobacter baumannii* were isolated from 196 patients, and the mean age of the 196 patients were 67.3 ± 11.5 years old (ranged from 31 to 81 years old). There were 115 (58.67%) males and 81 (41.33%) females from 196 selected patients (**Table 1**). Patients with *Acinetobacter baumannii* were selected from ICU, pneumology, emergency and cerebral surgery, accounting for 33.67%, 17.86%, 16.33% and 32.14%, respectively. 123 strains (86.62%) of clinical specimens were isolated from sputum, and 6 (4.23%) were isolated from cerebrospinal fluid.

Antimicrobial susceptibility of Acinetobacter baumannii

The antimicrobial susceptibility of 196 patients with *Acinetobacter baumannii* strain was shown in **Table 2**. Among the 196 patients with drug resistant strains, most strains showed resistance to different classes of agents, especially in ceftazidime, piperacillin, cefepime, nitrofurantoin and ertapenem.

Detection of OXA genes in Acinetobacter baumannii

The detection of *bla*OXA-51, *bla*OXA-23, *bla*OXA-24 and *bla*OXA-58 were shown in **Table 3** and **Figure 1**. Of 339 isolated strains, 164 (48.38%) were *bla*OXA-51, 157 (46.31%) were *bla*OXA-23, 18 (5.31%) were *bla*OXA-58, and no

strain for *bla*OXA-24. 143 (47.67%) strains of *bla*OXA-51, 143 (47.67%) strains of *bla*OXA-23, and 14 (4.66%) strains of *bla*OXA-58 showed multidrug-resistant. However, 21 (53.85%) stains of *bla*OXA-51, 14 (35.90%) strains of *bla*-OXA-23, and 4 (10.26%) strains of *bla*OXA-58 showed non-multidrug resistant.

Discussion

Acinetobacter baumannii causes a significant number of nosocomial outbreaks worldwide, which commonly occur in settings with high antibiotic selective pressures, such as ICUs. Most outbreak strains are highly resistant to antibiotics, and therefore therapeutic options are becoming increasingly limited [10]. Previous studies reported that *bla*OXA-23 and *bla*-OXA-51 are the most common detected genes in *Acinetobacter baumannii* [11]. *bla*OXA-24 in *Acinetobacter baumannii* was reported to be detected in Spain and Iran [12, 13]. *bla*OXA-58 was reported to be sequential outbreaks in a Saudia Arabia [14]. The present study finds that the main resistant genes in *Acinetobacter baumannii* were *bla*OXA-51 and *bla*OXA-23, and the main multidrug-resistant genes were found in ICU and pneumology departments.

Our study showed that the *Acinetobacter baumannii* presented multidrug resistant to different classes of agents, especially in ceftazidime, piperacillin, cefepime, nitrofurantoin and ertapenem. The main mechanism of drug resistant of *Acinetobacter baumannii* is due to four carbapenemases. One of the four carbapenemases includes *bla*OXA-23, *bla*OXA-24, *bla*OXA-51 and *bla*OXA-58. The OXA-51 is naturally existed and different with other acinetobacters [15].

In our study, 164 (48.38%) were *bla*OXA-51, 157 (46.31%) were *bla*OXA-23, 18 (5.31%) were *bla*OXA-58, and no strain for *bla*OXA-24. In a recent study, they detected the *bla*OXA-23 and *bla*OXA-58 in bacterial chromonsomes, but they also did not isolated strains of *bla*OXA-24 from patients [16]. The drug resistant rate of *Acinetobacter baumannii* to ceftazidime, piperacillin, cefepime, nitrofurantoin and ertapenem is above 90%. In a previous study, Vakili et al. reported that 95% of isolated strains showed multidrug resistant and 76.6% were high resistant, which were similar with the drug resistant with ours [17].

One previous study reported that the bla *bla*-OXA-51-like and bla *bla*OXA-23like were the main mechanisms of resistance to imipenem in *Acinetobacter baumannii* [12]. Another study in an Indian population reported that *bla*OXA-51-like and bla *bla*OXA-23like are the main pathogen for carbapenem-resistant Acinetobacter [18]. However, some studies did not reported similar results with ours. One study in a Spanish population reported that all isolates of multidrug-resistant *Acinetobacter baumannii* contained the *bla*OXA-51-like and *bla*OXA-58-like genes [19]. Another study in a Chinese population reported that *bla*OXA-23-like are the most frequent carbapenem-resistant *Acinetobacter baumannii* in China, and *bla*OXA-24 and *bla*OXA-58 gene have become the potential threats of hospital outbreaks of multidrug-resistant *Acinetobacter baumannii* [20]. The discrepancies between studies may be due to differences in samples selection and gene variations in different ethnicities as well as sample size.

In conclusion, our study found that *bla*OXA-51 and *bla*OXA-23 were the main mechanisms of resistant or sensitivity to carbapenems. Drug resistance is increasing in *Acinetobacter baumannii*, and thus the resistance surveillance has become increasingly important to prevent the spread of carbapenem resistant *Acinetobacter baumannii*.

Disclosure of conflict of interest

None.

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