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Analysis of drug-resistant gene detection of blaOXA-like genes from *Acinetobacter baumannii*

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ABSTRACT. Our study determines the resistance gene profile of a set of *Acinetobacter baumannii* hospital isolates. *A. baumannii* is responsible for nosocomial outbreaks and sporadic infections. We extracted and PCR amplified bacterial DNA isolated from patients with ages below 60 years (23.36%) and above 60 years (76.64%). Most of the patients were admitted in the ICU (36.13%) and pneumology departments (28.47%). Of 164 isolated strains, 16 (9.75%) contained OXA-51, 8 (4.88%) contained OXA-58, and 140 (85.37%) contained both OXA-51 and OXA-23. Additionally, 8 (7.41%) strains containing OXA-58 and 100 (92.59%) strains containing both OXA-51 and OXA-23 showed multidrug-resistance. Drug resistance rates of *A. baumannii* to amikacin, tobramycin-levofloxacin, and cotrimoxazole were above 90%, while drug resistance rates to ampicillin, cefotetan, cefazolin, cefoperazone, and nitrofurantoin were 100%. In conclusion, we found that isolated strains containing OXA-51 and OXA-23 were more likely to be resistant or have decreased sensitivity to carbapenems.

Key words: *Acinetobacter baumannii*; Drug-resistant gene; OXA-51; OXA-23; OXA-24; OXA-58

INTRODUCTION

Acinetobacter baumannii, an aerobic non-motile gram-negative coccobacillus, is an opportunistic pathogen, which is widely found in intensive care units (ICUs), and induces nosocomial infections such as pneumonia, septicemia, and urinary tract and wound infections. *A. baumannii* is frequently involved in outbreaks and can persist in the environment for several days (Metan et al., 2013). Previous studies reported that *A. baumannii* is the most common pathogenic bacteria isolated from hospitalized patients with pneumonia (Cefai et al., 1990; Lin et al., 2010). *A. baumannii* is usually found to be resistant to many drugs, including third-generation cephalosporins, aminoglycosides, and fluoroquinolone (Sinha and Srinivasa, 2007).

Multidrug-resistant *A. baumannii* strains can acquire antibiotic-resistance genes through class 1 integrons that carry single or multiple gene cassettes (Petersen et al., 2000). Integrons are genetic elements encoding antibiotic resistance genes that can integrate or mobilize their gene cassettes (Recchia and Hall, 1995). Aminoglycoside resistance genes, which are class 1 integrons, play an important role in the enzymatic inactivation of aminoglycoside antibiotics and could cause carbapenem resistance, such as bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SIM}, or $bla_{OXA\text{-}like}$ (Sung et al., 2008; Srinivasan et al., 2009). Carbapenemase production is the most well described mechanism of resistance to carbapenems (Poirel et al., 2007). The most common mechanism of drug resistance is associated with hydrolyzing β-lactamases of metallo-β-lactamases (Ambler class B) and oxacillinases (Ambler class D). In our study, we collected 131 strains to detect drug-resistance genes by PCR analysis and conducted homology analysis. We determined the resistance gene profile of *A. baumannii* responsible for nosocomial outbreaks and sporadic infections.

MATERIAL AND METHODS

Patients

In total, 274 *A. baumannii* isolates were collected from the First Affiliated Hospital of Xinxiang Medical University between May 2012 and May 2014. Strains were identified as *A. baumannii* by a PCR test using two amplification bands. We used 164 strains of *A. baumannii* to detect the distribution of genes.

Bacterial isolates and gene analysis

The DNA of bacteria was extracted using the TIANamp Bacteria DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China) and amplified using Taq PCR Master Mix (Shanghai Lifefeng Biotech Co., Ltd, Shanghai, China). Primers for OXA-51, OXA-23, OXA-24, and OXA-58 were produced by Shanghai Jierui Biological Engineering Co., Ltd. The primers for OXA-51, OXA-23, OXA-24, and OXA-58 were as follows: for OXA-51, TAATGCTTTGATCGGCCTTG (forward) and TGGATTGCACTTCATCTTGG (reverse); for OXA-23, ATGAATAAATATTTTACTTG (forward) and TTAAATAATATTCAGCTGTT (reverse); for OXA-24, TTCCCCTAACATGAATTTGT (forward) and GTACTAATCAAAGTTGTGAA (reverse); and for OXA-58, TTATCAAAATCCAATCGGC (forward) and TAACCTCAAACTTCTAATTC (reverse).

Each PCR mix was comprised of 25 µL Taq Mix, 1 µL primers, 1 µL template, and 50 µL RNase-Free dH_2O. The internal reference for OXA-51, OXA-23, OXA-24, and OXA-58 was OXA-

51. The PCR amplification was as follows: 5 min initial denaturation at 94°C, followed by 30 cycles of denaturation for 30 s at 95°C, annealing for 90 s at 72°C, and extension for 30 s at 72°C, followed by a final extension for 5 min at 72°C. PCR products were verified by gel electrophoresis using a 1.5% agarose gel and visualized using ethidium bromide staining. The 353-, 501-, 1024-, and 507-bp amplicons represented the OXA-51, OXA-23, OXA-24, and OXA-58 genes, respectively.

Statistical analysis

Frequencies were used to describe the distribution of categorical variables. Median and interquartile range was used for continuous variables. The association between *A. baumannii* and drug resistance was analyzed by the chi-square test. All P values were two sided, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Characteristics of 274 patients are shown in Table 1. Ages of 64 patients (23.36%) were below 60 years, while those of the remaining 210 patients (76.64%) were above 60 years. Most of the patients were in the ICU (36.13%) or pneumology departments (28.47). Of 164 strains of *A. baumannii*, 139 (84.76%) were clinical specimens isolated from sputum and 25 (15.24%) were isolated from cerebrospinal fluid, catheters, or pleural effusion.

Table 1. Characteristics of the patients included.

Variables	N = 274	%
Age (years)		
<60	64	23.36
≥60	210	76.64
Location of *Acinetobacter baumannii*		
ICU	99	36.13
Emergency	53	19.34
Cerebral surgery	44	16.06
Pneumology	78	28.47

Strains containing the drug resistant genes OXA-51, OXA-23, OXA-24, and OXA-58 are shown in Table 2 and Figure 1. Of 164 isolated strains, 16 (9.75%) contained OXA-51, 8 (4.88%) contained OXA-58, and 140 (85.37%) contained both OXA-51 and OXA-23. Additionally, 8 strains (7.41%) that contained OXA-58 and 100 (92.59%) strains that contained both OXA-51 and OXA-23 were multidrug-resistant. However, 16 (28.07%) strains containing OXA-51 and 40 (71.43%) strains containing both OXA-51 and OXA-23 were not multidrug-resistant.

Table 2. PCR results of drug-resistant genes.

Genes	Total	%	Multidrug-resistant	%	Non-multidrug resistant	%
OXA-51	16	9.75	0	0.00	16	28.07
OXA-23	0	0.00	0	0.00	0	0.00
OXA-24	0	0.00	0	0.00	0	0.00
OXA-58	8	4.88	8	7.41	0	0.00
OXA-51 + OXA-23	140	85.37	100	92.59	40	71.43

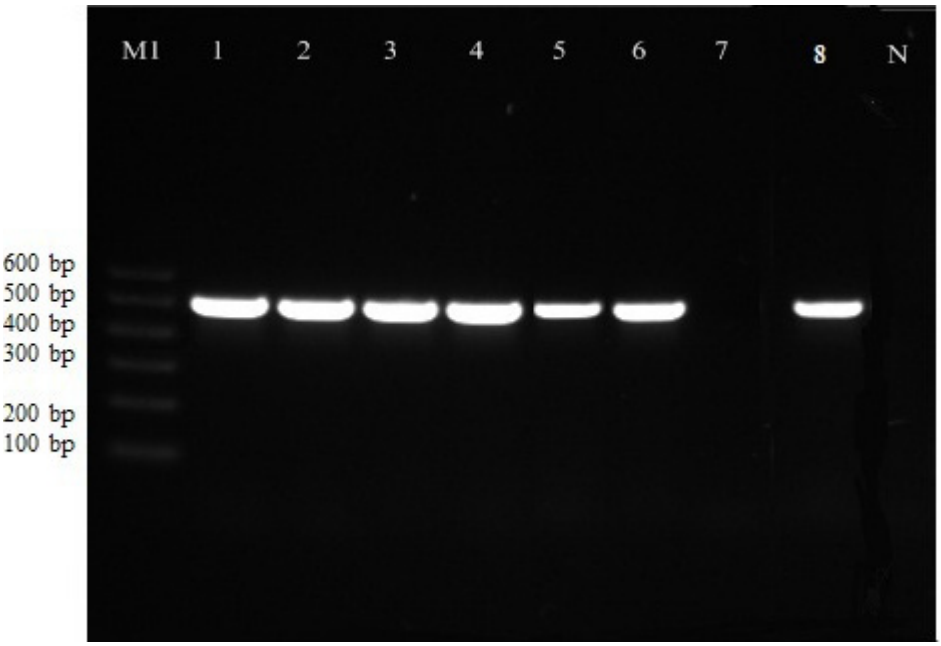

Figure 1. Gene PCR products of OXA-51, OXA-23, OXA-24, and OXA-58. *Lane MI* = markers; *lanes 1- 6,8* = OXA-23; *lane N* = negative control.

Additionally, we found that *A. baumannii* showed multidrug resistance to penicillin, quinolones, β-lactam antibiotics, cephalosporin, and carbapenems. Drug resistance rates of *A. baumannii* to amikacin, tobramycin-levofloxacin, and cotrimoxazole were above 90%, and drug resistance rates to ampicillin, cefotetan, cefazolin, cefoperazone, and nitrofurantoin were 100%.

DISCUSSION

A. baumannii causes a significant number of nosocomial outbreaks worldwide and commonly occurs 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 (Turton et al., 2006). We found that the main drug resistance genes were OXA-51 and OXA-23 in *A. baumannii* in isolates from the ICU and pneumology departments.

Our study also shows that the *A. baumannii* is resistant to penicillin, quinolones, β-lactam antibiotics, cephalosporin, and carbapenems. The main mechanism of drug resistance is due to four carbapenemases. One of the four carbapenemases includes OXA-23, OXA-24, OXA-51, and OXA-58. OXA-51 varies between different species of *Acinetobacter* (Durante-Mangoni and Zarrilli, 2011).

In our study, 16 (9.75%) strains contained OXA-51, 8 (4.88%) contained OXA-58, and 140 (85.37%) contained both OXA-51 and OXA-23. We did not detect OXA-23 and OXA-24 in any strains. In a recent study, OXA-23 and OXA-58 were detected on bacterial chromosomes, but strains isolated from patients did not contain OXA-24 (Li et al., 2010). Drug resistance rates of *A. baumannii* to amikacin, tobramycin levofloxacin, and cotrimoxazole were above 90%, while drug resistance rates to ampicillin, cefotetan, cefazolin, cefoperazone, and nitrofurantoin were 100%. In

a previous study, Vakili et al. (2014) reported that 95% of isolated strains show multidrug resistance and 76.6% were highly resistant, which is similar to our findings.

We found that OXA-51 and OXA-23 were the main antibiotic resistance genes found in *A. baumannii* isolates, similar to results of previous studies (Mohajeri et al., 2013; Chan et al., 2014; Dettori et al., 2014; Aly et al., 2014). Mohajeri et al. (2013) reported that the OXA-51 and OXA-23 were the predominant mechanisms of resistance to imipenem. Chan et al. (2014) reported that most of the isolated strains in their study contained OXA-23-like and OXA-51-like carbapenemase genes.

In conclusion, we found that isolated strains containing OXA-51 and OXA-23 were more likely to be resistant or have decreased sensitivity to carbapenems. Drug resistance is increasing in *A. baumannii,* and thus, resistance surveillance is becoming increasingly important to prevent the spread of carbapenem-resistant *A. baumannii*.

Conflicts of interest

The authors declare no conflict of interest.

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