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# Molecular characterization of carbapenemase genes in *Acinetobacter baumannii* in China

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**ABSTRACT.** *Acinetobacter baumannii* is an aerobic non-motile Gram-negative coccobacillus, and it is one of the most important nosocomial pathogens worldwide. The aim of this study was to determine the molecular epidemiology of the outbreak strains. Between March 2011 and March 2014, a total of 205 strains of *A. baumannii* were isolated from patients at the Nanyang City Center Hospital. The *bla*OXA-23, *bla*OXA-24, *bla*OXA-51, and *bla*OXA-58 genes were amplified by multiplex polymerase chain reaction. We found that 68 (33.17%) strains were positive for the *bla*OXA-23 gene, and 88.24% of these 68 showed resistance to carbapenems, while 11.76% were sensitive to carbapenems. The *bla*OXA-51 gene was found in 132 (64.39%) strains, and 17.42% of these were resistant to carbapenems while 82.58% were sensitive to carbapenems. Moreover, 5 (2.44%) strains were positive for *bla*OXA-58, of which 80% were resistant to carbapenems and 20% were sensitive to carbapenems. We found that *A. baumannii* showed 100% drug resistance to ampicillin, cefotetan, cefazolin, and cefoperazone. Our findings suggest that the *bla*OXA-23 and *bla*OXA-51

 

genes are most frequently identified in *A. baumannii*, while *bla*OXA-23 is the most important gene for resistance to carbapenems.

**Key words:** *bla*OXA-23; *bla*OXA-51; *Acinetobacter baumannii*; Carbapenems

## INTRODUCTION

*Acinetobacter baumannii* is an aerobic non-motile, Gram-negative coccobacillus that is regarded as one of the most important nosocomial pathogens worldwide (Boucher et al., 2009). *A. baumannii* is associated with many nosocomial infections, such as pneumonia, bloodstream infections, septicemia, and urinary tract and wound infections (Gaynes et al., 2005; Dijkshoorn et al., 2007). Multidrug-resistance in *A. baumannii* is on the rise in hospitals worldwide (Dijkshoorn et al., 2007; Peleg et al., 2008). Carbapenems are often used to treat multidrug-resistant *A. baumannii* infections; however, an increasing number of studies have reported the outbreak of carbapenem-resistant *A. baumannii*, thus creating a serious global therapeutic problem (Jeong et al., 2006; Yang et al., 2009; Peymani et al., 2012).

Carbapenem resistance arises through the recruitment and production of carbapenem-hydrolyzing class D β-lactamase (CHDLs) or metallo-β-lactamase. In *A. baumannii*, 4 subgroups of acquired CHDLs have been found, including *bla*OXA-23, *bla*OXA-24, *bla*OXA-51, and *bla*OXA-58 (Poirel and Nordmann, 2006). Although these enzymes are weak hydrolyzers of carbapenems, drug resistance can develop through overexpression of the *bla*OXA genes. The aim of this study is to determine the molecular epidemiology of the outbreak strains.

## MATERIAL AND METHODS

Between March 2011 and March 2014, a total of 205 strains of *A. baumannii* were isolated from infected patients at the Nanyang City Center Hospital. The strains were collected from patients who did not receive antibiotic therapy. All the samples were collected and stored at -80°C.

### DNA extraction and gene analysis

The DNA of *A. baumannii* was extracted using the TIANamp Bacteria DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China). Polymerase chain reaction (PCR) amplification was performed using Taq PCR Master Mix (Shanghai Lifefeng Biotech Co., Ltd., Beijing, China). The *bla*OXA-23, *bla*OXA-24, *bla*OXA-51, and *bla*OXA-58 genes were amplified by multiplex PCR. The primer sequences are listed in Table 1.

**Table 1.** PCR primers for PCR.

| OXA gene | Primer | Sequence | Amplicon size (bp) |
|---|---|---|---|
| *bla*OXA-23 | OXA-23-like F | GATCGGATTGGAGAACCAGA | 501 |
| | OXA-23-like R | ATTTCTGACCGCATTTCCAT | |
| *bla*OXA-24 | OXA-24-like F | TTCCCCTAACATGAATTTGT | 1024 |
| | OXA-24-like R | GTACTAATCAAAGTTGTGAA | |
| *bla*OXA-51 | OXA-51-like F | TAATGCTTTGATCGGCCTTG | 353 |
| | OXA-51-like R | TGGATTGCACTTCATCTTGG | |
| *bla*OXA-58 | OXA-58-like F | TGGCACGCATTTAGACCG | 507 |
| | OXA-58-like R | AAACCCACATACCAACCC | |

 

The PCRs were carried out in a total reaction volume of 50 μL, consisting of 25 μL Taq Mix, 1 μL primers, 1 μL DNA template, and 22 μL RNase-free $H_2O$. OXA-51 and 16S rRNA were used as internal controls. The cycling conditions were as follows: an initial denaturation step of 8 min at 94°C, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. The purity and integrity of each PCR product was evaluated after separation on 3% agarose gel electrophoresis and analysis under ultraviolet light.

### Antibiotic susceptibility testing

The antibiotic susceptibility of the *A. baumannii* strains was assessed using a Vitek 2 Compact system (bioMérieux, Inc., Marcy-l'Etoile, France). Three strains were used as control strains, including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 29213).

### Statistical analysis

Frequencies were used to describe the distribution of categorical variables and median and interquartile ranges were used for continuous variables. All tests were two-sided and $P < 0.05$ was considered significant. Statistical analysis was conducted using the SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Strain identification

Of the 205 *A. baumannii* strains that we collected in total, 130 (63.41%) were isolated from the intensive care unit, 60 (29.27%) from surgical wards, and 15 (7.32%) from general wards. The samples were obtained from various infection sites: 141 (68.78%) strains were isolated from sputum, 23 (11.22%) from the nose, 22 (10.74%) from blood, 14 (6.83%) from wounds, and the remaining 6 (2.44%) were isolated from other sites.

### Detection of carbapenem-resistance genes

By multiplex PCR, we found that 68 (33.17%) strains were positive for the *bla*OXA-23 gene. Of these trains, 88.24% showed resistance to carbapenems while 11.76% were sensitive to carbapenems (Figure 1). The *bla*OXA-51 gene was identified in 132 (64.39%) strains, and 17.42% of these were resistant to carbapenems, while 82.58% showed sensitivity to carbapenems. In addition, 5 (2.44%) strains were positive for *bla*OXA-58. Only 80% of these strains showed resistance to carbapenems and 20% were sensitivity to carbapenems (Table 2). Moreover, the *bla*OXA-24 was not detected by multiplex PCR analysis.

### Antibiotic susceptibility testing

We tested the antibiotic susceptibility of the 205 isolated *A. baumannii* strains. We found that *A. baumannii* was 100% resistant to ampicillin, cefotetan, cefazolin, and cefoperazone (Table 3). Moreover, we found that *A. baumannii* exhibited a high resistance (>50%) to cefepime,

piperacillin-tazobactam, amikacin, levofloxacin, ciprofloxacin, tetracycline, ceftazidime, cefotaxime, sulfamethoxazole-trimethoprim, gentamicin, and piperacillin. The drug resistance to cefoperazone-sulbactam, meropenem, and imipenem was lower.

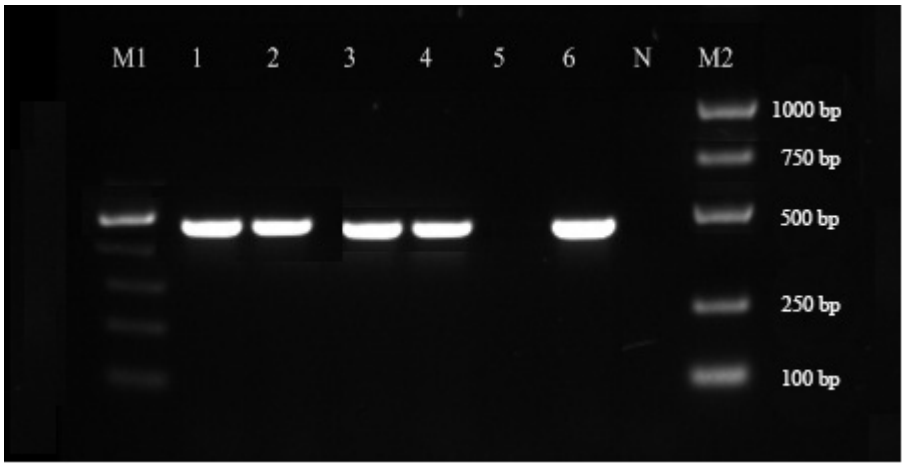


**Figure 1.** Positive *bla*OXA-23 gene of PCR test. *Lanes 1-4* and *6* = *bla*OXA-23; *lanes M1* and *M2* = molecular markers; *lane N* = negative control.

**Table 2.** PCR results for carbapenem-resistance genes.

| OXA genes | Positive PCR | Carbapenem resistant | | Carbapenem sensitive | |
|---|---|---|---|---|---|
| | N | N | % | N | % |
| blaOXA-23 | 68 | 60 | 88.24 | 8 | 11.76 |
| blaOXA-24 | 0 | 0 | 0.00 | 0 | 0.00 |
| blaOXA-51 | 132 | 23 | 17.42 | 109 | 82.58 |
| blaOXA-58 | 5 | 4 | 80.00 | 1 | 20.00 |

**Table 3.** *Acinetobacter baumannii* drug resistance.

| Antibiotic drug | N | % |
|---|---|---|
| Ampicillin | 205 | 100.00 |
| Cefoperazone-sulbactam | 13 | 6.34 |
| Cefotetan | 205 | 100.00 |
| Cefazolin | 205 | 100.00 |
| Meropenem | 46 | 22.44 |
| Imipenem | 66 | 32.20 |
| Cefepime | 142 | 69.27 |
| Piperacillin-tazobactam | 167 | 81.46 |
| Amikacin | 165 | 80.49 |
| Levofloxacin | 154 | 75.12 |
| Ciprofloxacin | 172 | 83.90 |
| Tetracycline | 174 | 84.88 |
| Ceftazidime | 178 | 86.83 |
| Cefotaxime | 179 | 87.32 |
| Cefoperazone | 205 | 100.00 |
| Sulfamethoxazole-trimethoprim | 173 | 84.39 |
| Gentamicin | 190 | 92.68 |
| Piperacillin | 198 | 96.59 |

## DISCUSSION

The non-fermenting, aerobic bacillus *A. baumannii* is an opportunistic pathogen that causes nosocomial infections, especially in intensive care units. *A. baumannii* easily spreads from patient to patient in hospitals, for example via medical equipment, and the infection is hard to control because of high drug resistance (Bergogne-Bérézin and Towner, 1996; Abbo et al., 2005). Carbapenems are generally used to treat patients suffering from *A. baumannii* infections, as they are currently the most active antibiotics. However, through the production of carbapenemase and the acquisition of carbapenem-hydrolyzing β-lactamases of the Ambler class B and D (Poirel and Nordmann, 2006; Queenan and Bush, 2007), *A. baumannii* can acquire resistance to carbapenems as well. The *bla*OXA-23, *bla*OXA-24, *bla*OXA-51, and *bla*OXA-58 genes encode four Ambler class D β-lactamases, and the present study investigated their role in the drug resistance of *A. baumannii*.

We found that the *bla*OXA-23- and *bla*OXA-51-resistance genes were most frequently present in *A. baumannii* isolates, and that *bla*OXA-23 is mainly responsible for resistance to carbapenems. Previous studies have reported similar results (Jeannot et al., 2014; Santimaleeworagun et al., 2014; Khorsi et al., 2015; Mathlouthi et al., 2015; Memish et al., 2015; Wang et al., 2015). Khorsi et al. (2015) analyzed the prevalence of *A. baumannii* multidrug-resistance in hospitals in Algiers, and found that carbapenem resistance was mainly mediated by *bla*OXA-23 and *bla*OXA-24 genes. Wang et al. (2015) also identified the *bla*OXA-23 gene in multidrug-resistant *A. baumannii* isolates from Libyan hospitals. Memish et al. (2015) have reported that *bla*OXA-23 is the dominant carbapenemase in *A. baumannii*. Jeannot et al. (2014) reported that *bla*OXA-23 was the most frequently acquired gene in *A. baumannii.* In contrast with these findings, Ma et al. (2015) reported that *bla*OXA51 and *bla*OXA58 genes were most frequently detected in *A. baumannii*. These authors did not identify *bla*OXA-23 in samples collected at a Chinese hospital. The discrepancies between the findings in these studies may be explained by differences in sample selection or by genetic variation among the different populations.

Our study shows that 100% of the *A. baumannii* isolated strains is resistant to ampicillin, cefotetan, cefazolin, and cefoperazone. In addition, *A. baumannii* is highly resistant to cefepime, piperacillin-tazobactam, amikacin, levofloxacin, ciprofloxacin, tetracycline, ceftazidime, cefotaxime, sulfamethoxazole-trimethoprim, gentamicin, and piperacillin. Previous studies have reported similar results (Vakili et al., 2014; Ma et al., 2015; Zhao et al., 2015). Zhao et al. (2015) reported that the most important drug resistance genes of *A. baumannii* are *bla*OXA-51 and *bla*OXA-23, conferring multidrug resistance to the bacterial strain.

In summary, our study suggests that the *bla*OXA-23 and *bla*OXA-51 genes are most frequently present in *A. baumannii*, and that resistance to carbapenems is mainly correlated with *bla*OXA-23 gene expression. Further molecular analysis of *A. baumannii* could help to identify common sources of infection, and to prevent its spread in hospitals.

### Conflicts of interest

The authors declare no conflict of interest.

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