



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

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ABSTRACT. We distinguished the four OXA-type carbapenemase subgroup alleles present in 120 strains of *Acinetobacter baumannii* by using polymerase chain reaction (PCR) and investigated the distributions of the OXA subgroups in clinically isolated samples. Amplification of the OXA genes *bla*OXA-23, *bla*OXA-24, *bla*OXA-51, and *bla*OXA-58 was performed by multiplex PCR. Antibiotics susceptibility test was conducted for determine the sensitivity of the *A. baumannii* to clinical common used antibiotics by Kirby-Bauer method. Results revealed that 46 (51.69%) of the samples were positive for only the *bla*OXA51 gene and 41 (46.07%) were positive for both the *bla*OXA51 and *bla*OXA58 genes in the 89 isolates of *A. baumannii*. Among these, 45 were carbapenem-resistant and 44 carbapenem-sensitive. Strains containing either *bla*OXA51 or *bla*OXA58 showed resistance or sensitivity to carbapenems, respectively. *A. baumannii* isolated from intensive care units showed significantly higher resistance rate to Cefepime, Piperacillin-tazobactam, Amikacin,

Ceftazidime, Cefotaxime, Sulfamethoxazole-trimethoprim, and Gentamicin than those isolated from other departments ($P < 0.05$). In conclusion, we found that the presence of blaOXA-51 and blaOXA-58 appears to convey a mechanism of resistance or sensitivity to carbapenems, respectively, in *A. baumannii* clinical isolates.

Key words: Genomic diversity; OXA; *Acinetobacter baumannii*

INTRODUCTION

Acinetobacter baumannii is an aerobic non-motile gram-negative coccobacillus, and it is considered one of the major nosocomial pathogens worldwide. This polymorphic bacterial pathogen easily spreads between patients and can persist in the environment for several days (Metan et al., 2013). It is well known that *A. baumannii* is the most common pathogenic bacteria isolated from hospitalized patients with pneumonia, other respiratory tract infections, bloodstream infections, urinary tract infections and surgical site infections, and it has an important role in nosocomial infections (Cefai et al., 1990; Lin et al., 2010).

It is reported that *Acinetobacter* is a common nosocomial pathogen, which is widely observed in intensive care units (ICUs), causing severe infections (Fournier and Richet, 2006). Typically, isolates of *A. baumannii* usually present multidrug resistance, including resistance to third generation cephalosporins, aminoglycosides, and fluoroquinolone (Sinha and Srinivasa, 2007). Previous experimental study has reported that the most common mechanism of drug resistance is the presence of hydrolyzing β -lactamases, including metallo- β -lactamases (Ambler class B) and oxacillinases (Ambler class D) (Soares et al., 2012). The widespread use of antimicrobial chemotherapy has played an important role in the appearance of carbapenem-hydrolyzing class D β -lactamases (CHDLs), which have been widely identified in *A. baumannii*. Four common subgroups of acquired CHDLs are reported in *A. baumannii*, including blaOXA-23, blaOXA-24, blaOXA-51, and blaOXA-58. Here, we set out to distinguish the four OXA subgroup alleles in 120 samples of *A. baumannii* by using multiplex polymerase chain reaction (PCR) and to investigate the distributions of the OXA subgroups in these clinical isolates and the results of antimicrobial susceptibility test.

MATERIAL AND METHODS

In total, 120 *A. baumannii* samples were isolated from Shenzhen Longgang Central Hospital between 2010 and 2013. The isolates were identified as *A. baumannii* by a multiple PCR test. The presence of two amplification bands was characteristic of *A. baumannii*. This process allowed 120 samples of *A. baumannii* to be identified, and we randomly selected 89 of these to examine the distributions of OXA gene alleles.

PCR amplification

Amplification of OXA genes, blaOXA-23, blaOXA-24, blaOXA-51, and blaOXA-58, was performed by multiplex PCR. The primers for blaOXA-23, blaOXA-24, blaOXA-51, and blaOXA-58 utilized were as follows: for blaOXA-23, the forward and reverse primers were 5'-GATCGGATTGGAGAACCAGA-3' and 5'-ATTTCTGACCGCATTTCAT-3', respectively; for

*bla*OXA-24, the forward and reverse primers were 5'-GGTTAGTTGGCCCCCTTAAA-3' and 5'-AGTTGAGCGAAAAGGGGATT-3', respectively; for *bla*OXA-51, the forward and reverse primers were 5'-TAATGCTTTGATCGGCCTTG-3' and 5'-TGGATTGCACTTCATCTTGG-3', respectively; for *bla*OXA-58, the forward and reverse primers were 5'-AAGTATTGGGGCTTGTGCTG-3' and 5'-CCCCTCTGCGCTCTACATAC-3', respectively.

Culturing of *A. baumannii*

The isolates of *A. baumannii* were kept at -70°C until use. Pure cultures of *A. baumannii* were recovered on Mueller-Hinton agar, incubated in a constant temperature incubator at 37°C. A single colony was cultured in Mueller-Hinton broth (MHB) for 12-16 h at 37°C in a constant temperature incubator, with shaking at 180 rpm.

The genomic DNA extraction from all *A. baumannii* isolates was performed with the pure fast bacterial genomic DNA purification kit (Tiangen, Beijing, China). Each PCR reaction mix comprised 50 ng genomic DNA, 200 µM dNTPs, 2.5 U Taq DNA polymerase (Promega, Madison, WI, USA), and 200 µM primers, in a total volume of 20 µl. The PCR reactions were initially denatured 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 separated by 1% agarose gel electrophoresis and visualized using ethidium bromide staining and UV light. Reproducibility was verified by repeating the analysis of a randomly chosen subgroup comprising 10% of the subject samples.

Antimicrobial susceptibility test

Antibiotics susceptibility test was conducted for determine the sensitivity of the *A. baumannii* to clinical common used antibiotics by Kirby-Bauer method (Oxoid Company, Basingstoke, UK). The results were determined by criteria from Clinical and Laboratory Standards Institute (CLSI). The *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 29213) were used as quality control bacteria.

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. Differences between categorical variables were compared by χ^2 -test. All P values were two sided, and a P value < 0.05 was considered statistically significant.

RESULTS

Among the 120 samples, 89 were identified as *A. baumannii* and genotyped for the distribution of the OXA gene alleles. Among the isolated strains, 67 strains (69.79%) were isolated from intensive care units, and 29 (30.21%) were from other departments. Of the 89 isolates, 58 (65.17%) were isolated from male patients, 31 (34.83%) were from females, 70 (78.65%) were from patients with hospital stays >4 weeks.

Multiplex PCR results revealed that of the 89 isolates, 46 (51.69%) were positive for only the *bla*OXA51 gene, whereas 41 (46.07%) were positive for both *bla*OXA51 and *bla*OXA58 genes. Two samples (2.25%) were observed to be positive for both *bla*OXA51 and *bla*OXA23 genes.

However, none of the isolates was positive for the *bla*OXA-23 and *bla*OXA-24 combination.

Moreover, we examined resistance to carbapenems in the *A. baumannii* isolates (Table 1). Among the 89 samples, 45 strains (50.56%) appeared resistant to carbapenems and 44 strains (49.44%) showed sensitivity to carbapenems. Among the 46 isolates positive for *bla*OXA51, we found 8 isolates (8.99%) with resistance to carbapenems and 38 isolates (42.70%) with apparent sensitivity to carbapenems. Of the 41 isolates with both *bla*OXA51 and *bla*OXA58, 36 (40.45%) showed resistance to carbapenems, while 5 (5.62%) were sensitive to carbapenems. Of the two strains with both *bla*OXA51 and *bla*OXA23, one strain showed resistance (1.12%) and the other showed sensitivity to carbapenems (1.12%).

By Kirby-Bauer method, we compared the drug resistance situation of 89 strains between intensive care units and other departments (Table 2). *A. baumannii* isolated from intensive care units showed significantly higher resistance rate to Cefepime, Piperacillin-tazobactam, Amikacin, Ceftazidime, Cefotaxime, Sulfamethoxazole-trimethoprim and Gentamicin than those isolated from other departments ($P < 0.05$).

Table 1. Distribution of *bla*OXA genes in *Acinetobacter baumannii* clinical isolates and their carbapenem resistance profiles.

PCR-positive for genes	Resistant to carbapenems (%)	Sensitivity to carbapenems (%)
OXA23	0 (0)	0 (0)
OXA24	0 (0)	0 (0)
OXA51	8 (8.99)	41 (42.70)
OXA58	0 (0)	0 (0)
OXA51+ OXA58	38 (40.45)	6 (5.62)
OXA51+ OXA23	1 (1.12)	1 (1.12)

Table 2. Drug resistance situation between groups.

Antibiotic drugs	Resistance rate (%)		χ^2 -test	P value
	In intensive care units	In other departments		
Cefoperazone-sulbactam	5.62	3.37	0.26	0.61
Meropenem	21.35	10.11	1.55	0.21
Imipenem	26.97	10.11	3.23	0.07
Cefepime	67.42	38.20	7.12	0.01
Piperacillin-tazobactam	76.40	48.31	7.18	0.01
Amikacin	78.65	51.69	7.34	0.01
Levofloxacin	77.53	61.80	2.48	0.12
Ciprofloxacin	80.90	68.54	1.55	0.21
Tetracycline	83.15	68.54	2.62	0.11
Ceftazidime	85.39	58.43	8.02	0.01
Cefotaxime	86.52	61.80	7.36	0.01
Sulfamethoxazole-trimethoprim	89.89	71.91	4.51	0.03
Gentamicin	91.01	71.91	5.64	0.02
Piperacillin	95.51	86.52	2.6	0.11

DISCUSSION

Multi-drug resistant *A. baumannii* has caused many nosocomial outbreaks worldwide, and it is considered as a troublesome nosocomial pathogen. Most outbreaks caused by *A. baumannii* present highly resistant to antibiotics, and thus the treatments has become limited (Turton et al., 2006). It is reported that acquired OXA carbapenemases has the main spread of OXA enzymes worldwide. Four common genetic subgroups of acquired CHDLs are reported in *A. baumannii*,

including *bla*OXA-23, *bla*OXA-24, *bla*OXA-51 and *bla*OXA-58 (Kusradze et al., 2011; Mohajeri et al., 2013; Cayô et al., 2014; Aly et al., 2014).

In our study, we only detected positive samples for *bla*OXA-51 and *bla*OXA-58, known to be distributed globally among *A. baumannii* isolates; furthermore, we found that *bla*OXA-51 and *bla*OXA-58 appear to be important in conferring resistance or sensitivity to carbapenems, respectively. In the present study, we found that 46 (51.69%) were positive for only the *bla*OXA51 gene, whereas 41 (46.07%) were positive for both *bla*OXA51 and *bla*OXA58 genes. Two samples (2.25%) were observed to be positive for both *bla*OXA51 and *bla*OXA23 genes. The results of our study show similarities with those of previous studies (Ciftci et al., 2010; Amudhan et al., 2011; Bali et al., 2013; Mohajeri et al., 2013). In a recent study, Ciftci et al. (2010) identified the frequencies of *bla*OXA23, *bla*OXA51 and *bla*OXA58 genes in carbapenem-resistant *A.baumannii* clinical isolates. Amudhan et al. (2011) reported that *bla*OXA-23 and *bla*OXA 51 genes are the most common types of OXA carbapenamases. Bali et al. (2013) reported that *bla*OXA-23 and *bla*OXA-51 were the major factors underlying the pathogenicity for carbapenem-resistant *Acinetobacter*. Mohajeri et al. (2013) reported that *bla*OXA-51-like and *bla*OXA-23-like genes were the predominant mechanisms of resistance to imipenem in *A. baumannii*. However, certain results of previous studies appear to be inconsistent with those of our study (Jeannot et al., 2014; Ji et al., 2014; Xia et al., 2014; Mohajeri et al., 2015). One study reported that only *bla*OXA-23 and *bla*OXA-51 were amplified in 96.7% of the *A. baumannii* strains, while neither *bla*OXA-24 nor *bla*OXA-58 was amplified (Xia et al., 2014). Jeannot et al. (2014) reported that *bla*OXA-23 and *bla*OXA-24 were the most frequently detected genes in isolated *A. baumannii*. Ji et al. (2014) reported that *bla*OXA-24 and *bla*OXA-58 have emerged as potential threats for hospital outbreaks of multidrug-resistant *A. baumannii*. Mohajeri et al. (2015) observed that *bla*OXA-23-like gene were the main genes of *A. baumannii*. for high resistant to anti-microbial agents. The discrepancies between the studies may be due to differences in sample selection or genetic variation among the different ethnicities examined, as well as the sample size used for the study. Therefore, further studies are pressingly needed to confirm our findings.

Our study found that the *A. baumannii* isolated from intensive care units showed significantly higher resistance rate to Cefepime, Piperacillin-tazobactam, Amikacin, Ceftazidime, Cefotaxime, Sulfamethoxazole-trimethoprim and Gentamicin than those isolated from other departments. The main mechanism of drug resistant from *A. baumannii* is caused by the four carbapenamases. OXA-23, OXA-24, OXA-51, and OXA-58 belongs to the carbapenamases.

In summary, we have suggested that the presence of *bla*OXA-51 and *bla*OXA-58 is the main mechanism of resistance or sensitivity to carbapenems, respectively. Both phenotyping and genotyping methods are important for the detection of carbapenem resistance in *Acinetobacter*.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

- Alvargonzalez JJ, Vindel HA, Martin MD, Casas CM, et al. (2014). Sequential outbreaks in a Spanish hospital caused by multidrug-resistant OXA-58-producing *Acinetobacter baumannii* of ST92. *J. Med. Microbiol.* 63: 1093-1098.
- Aly M, Tayeb HT, Al Johani SM, Alyamani EJ, et al. (2014). Genetic diversity of OXA-51-like genes among multidrug-resistant *Acinetobacter baumannii* in Riyadh, Saudi Arabia. *Eur. J. Clin. Microbiol. Infect. Dis.* 33: 1223-1228.
- Bali NK, Fomda BA, Bashir H, Zahoor D, et al. (2013). Emergence of carbapenem-resistant *Acinetobacter* in a temperate north Indian State. *Br. J. Biomed. Sci.* 70: 156-160.

- Cayó R, Merino M, Ruiz Del Castillo B, Cano ME, et al. (2014). OXA-207, a novel OXA-24 variant with reduced catalytic efficiency against carbapenems in *Acinetobacter pittii* from Spain. *Antimicrob. Agents. Chemother.* 58: 4944-4948.
- Cefai C, Richards J, Gould FK and McPeake P (1990). An outbreak of *Acinetobacter* respiratory tract infection resulting from incomplete disinfection of ventilatory equipment. *J. Hosp. Infect.* 15: 177-182.
- Fournier PE and Richet H (2006). The epidemiology and control of *Acinetobacter baumannii* in health care facilities. *Clin. Infect. Dis.* 42: 692-699.
- Jeannot K, Diancourt L, Vaux S, Thouverez M, et al (2014). Molecular epidemiology of carbapenem non-susceptible *Acinetobacter baumannii* in France. *PLoS One.* 9: e115452.
- Ji S, Chen Y, Ruan Z, Fu Y, et al. (2014). Prevalence of carbapenem-hydrolyzing class D β -lactamase genes in *Acinetobacter* spp. isolates in China. *Eur. J. Clin. Microbiol. Infect. Dis.* 33: 989-997.
- Kusradze IA, Diene SM, Goderdzishvili M and Rolain JM (2011). Molecular detection of OXA carbapenemase genes in multidrug-resistant *Acinetobacter baumannii* isolates from Iraq and Georgia. *Int. J. Antimicrob. Agents.* 38: 164-168.
- Lin YC, Sheng WH, Chen YC, Chang SC, et al. (2010). Differences in carbapenem resistance genes among *Acinetobacter baumannii*, *Acinetobacter* genospecies 3 and *Acinetobacter* genospecies 13TU in Taiwan. *Int. J. Antimicrob. Agents.* 35: 439-443.
- Metan G, Sariguzel F, Sumerkan B, Reijden TY, et al. (2013). Clonal diversity and high prevalence of OXA-58 among *Acinetobacter baumannii* isolates from blood cultures in a tertiary care centre in Turkey. *Infect. Genet. Evol.* 14: 92-97.
- Mohajeri P, Farahani A, Feizabadi MM, Ketabi H, et al. (2013). Antimicrobial susceptibility profiling and genomic diversity of *Acinetobacter baumannii* isolates: A study in western Iran. *J. Microbiol.* 5: 195-202.
- Mohajeri P, Farahani A, Feizabadi MM, Norozi B (2015). Clonal evolution multi-drug resistant *Acinetobacter baumannii* by pulsed-field gel electrophoresis. *Indian. J. Med. Microbiol.* 33: 87-91.
- Sinha M and Srinivasa H (2007). Mechanisms of resistance to carbapenems in meropenem-resistant *Acinetobacter* isolates from clinical samples. *Ind. J. Med. Microbiol.* 25: 121-125.
- Soares GM, Figueiredo LC, Faveri M, Cortelli SC, et al. (2012). Mechanisms of action of systemic antibiotics used in periodontal treatment and mechanisms of bacterial resistance to these drugs. *J. Appl. Oral. Sci.* 20: 295-309.
- Turton JF, Ward ME, Woodford N, Kaufmann ME, et al. (2006). The role of ISAba1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol. Lett.* 258: 72-77.
- Xia J, Zhang D, Xu Y, Gong M, et al. (2014). A retrospective analysis of carbapenem-resistant *Acinetobacter baumannii*-mediated nosocomial pneumonia and the *in vitro* therapeutic benefit of cefoperazone/sulbactam. *Int. J. Infect. Dis.* 23: 90-93.