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Molecular characterization of oxacillinases among carbapenem-resistant *Acinetobacter baumannii* nosocomial isolates in a Saudi hospital

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Summary
Background: *Acinetobacter baumannii* has successfully become a significant nosocomial pathogen because of its remarkable ability to acquire antibiotic resistance and to survive in nosocomial environments. This study aimed to determine the drug susceptibility patterns and the distribution of four subgroups of carbapenem-hydrolyzing class D β-lactamases (OXA-carbapenemases), as well as their insertion sequences (IS*Aba1*), among *A. baumannii* nosocomial isolates from a Saudi tertiary care hospital.
Methods: A total of 108 non-duplicate *A. baumannii* isolates were identified, and their susceptibilities to different antibiotics were determined using the breakpoint method. Isolates were then subjected to multiplex-PCR targeting bla_{OXA} genes.

Abbreviations: OXA-carbapenemases, carbapenem hydrolyzing class D β-lactamases; IS*Aba1*, insertion sequence.

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Results: More than 75% of the isolates showed resistance to different antibiotics. The rates of susceptibility to colistin, meropenem, imipenem and trimethoprim—sulfamethoxazole were 95.6, 50, 48.1 and 34.3%, respectively. All isolates possessed a $bla_{\text{OXA-51-like}}$ gene. Of the 56 carbapenem-resistant isolates, 48 isolates (85.7%) carried $bla_{\text{OXA-23-like}}$, 3 isolates (5.4%) carried $bla_{\text{OXA-40-like}}$ and two isolates (3.6%) had $bla_{\text{OXA-58-like}}$ genes. The IS*Aba1* element was found upstream of the $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-24}}$ genes in 40 (71.4%) and 3 (5.4%) isolates, respectively, while it was detected upstream of $bla_{\text{OXA-51}}$ in only one (1.8%) isolate.
Conclusion: Our findings further illustrate the challenge of increasing carbapenem-resistance in *A. baumannii* isolates in Saudi Arabia. The high distribution of class D carbapenemase-encoding genes, mainly IS*Aba1*/OXA-23 and IS*Aba1*/OXA-24 carbapenemases, is worrisome and presents an emerging threat in our hospital. Local molecular surveillance is essential to help control carbapenem-resistant *A. baumannii* nosocomial infections and to prevent DNA exchange among endemic nosocomial pathogens.

Introduction

Acinetobacter baumannii is an important opportunistic pathogen and is often involved in various nosocomial infections, such as bacteremia, urinary tract infection, secondary meningitis, surgical site infection, and nosocomial and ventilator-associated pneumonia, especially in patients admitted to intensive care and burn units [1]. *A. baumannii* is notorious for its remarkable innate and acquired resistance to multiple antimicrobial classes, including extended-spectrum cephalosporins and carbapenems. Resistance to carbapenems is the most concerning, as carbapenems have a potent activity against *Acinetobacter* spp and are often used as a last resort for the treatment of infections due to multi-resistant *A. baumannii* isolates. The emergence of carbapenem-resistant *A. baumannii* has been described as the sentinel event of antimicrobial resistance [2,3]. Reports from different regions in Saudi Arabia have shown imipenem (IMP) resistance rates of 2.6—13.1% [4,5].

Carbapenem resistance in *A. baumannii* can be mediated by various mechanisms, including impermeability due to the loss of one of its major porins and, possibly, efflux, as shown recently for meropenem [1,6]. Most frequently, though, it is mediated through the enzymatic hydrolysis of the drug, particularly by carbapenem-hydrolyzing class D β-lactamases (oxacillinases). Oxacillinases can be grouped into six subclasses: chromosomal OXA-51-like, acquired OXA-23-like (OXA-23, OXA-27 and OXA-49), OXA-24/40-like (OXA-24, OXA-25, OXA-26, OXA-40 and OXA-72), OXA-58-like, OXA-143-like, and OXA-235-like (OXA-235, OXA-236 and OXA-237) β-lactamases [7,8]. Although they are weak carbapenem hydrolysers, they confer resistance when over-expressed as a result of their association with mobile elements, such as IS*Aba*1, which carries a strong promoter [9].

Carbapenem resistance due to OXA-carbapenemases has been reported in diverse geographical regions [1,10—12]. There is little information on the prevalence and distribution of β-lactamases in *A. baumannii* from Saudi Arabia, although resistance is frequent. However, few studies have reported the frequent isolation of OXA-23-producing *A. baumannii* in Saudi Arabia, suggesting that $bla_{\text{OXA-23}}$-carrying *A. baumannii* strains have become endemic [13—15]. Conversely, reports concerning the $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ genes are limited to the description of sporadic isolates [13—15].

The present study aimed to determine the drug susceptibility patterns and the distribution of four subgroups of OXA-carbapenemases and the insertion sequence, IS*Aba1*, among *A. baumannii* nosocomial isolates from a Saudi tertiary care hospital over a 6-month period.

Materials and methods

This study was conducted at Aseer Central Hospital (Abha, Saudi Arabia), which is a 500-bed tertiary care hospital located in Abha City, over a 6-month period from October 2013 to March 2014. Non-duplicate *A. baumannii* strains were isolated from blood, urine, respiratory secretions (sputum and tracheal aspirate), stool, pus, throat swabs, ascetic fluid and the tips of central venous catheters. The study included different patients who were hospitalized for ≥48 h and classified according to the Centers for Disease Control and Prevention/National Healthcare Safety Network (CDC/NHSN) criteria [16]. The strains were identified using conventional biochemical tests [17], the API 20NE (Biomerieux) and MicroScan Walkaway automated systems (Dade Behring, CA), according to the manufacturers' instructions.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed with the breakpoint method, using the MicroScan Walkaway automated systems, and the E-test minimum inhibitory concentration method, using E-test strips (AB Biodisk, Slona, Sweden) on Mueller—Hinton agar plates per the guidelines of the Clinical and Laboratory Standards Institute [18]. The antibiotics tested included amikacin, ceftazidime, cefotaxime, cefepime, gentamicin, tobramycin, imipenem, meropenem (MEM), piperacillin, ciprofloxacin, levofloxacin, trimethoprim—sulfamethoxazole and colistin.

All isolates (one isolate/patient) were sent to the microbiology department of the Najran University College of Medicine for molecular analysis.

Identification of the oxacillinases genes

All isolates were subjected to multiplex PCR to detect $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$ and $bla_{\text{OXA-58-like}}$ genes, as previously reported [19]. All primers used in this study are listed in Table 1. PCR was carried out in a thermocycler (Cyclogene, Techne, UK). A single reaction mixture contained: 30 ng of genomic DNA, 20 pM of each primer, 10 μl of reaction buffer, 3 μl of 25 mM $MgCl_2$, 1 μl of dNTPs and 0.25 μl of go Taq Polymerase (Promega, USA), with a final volume of 50 μl. Initial denaturation (94 °C for 3 min) was followed by 30 cycles of amplification. Each cycle consisted of 94 °C for 25 s, 52 °C for 40 s, and 72 °C for 50 s. A final extension step (72 °C for 5 min) completed the amplification.

Table 1 Sequences of primers used in this study for multiplex PCR for detection of genes encoding oxacillinases in *A. baumannii* isolates [9,19].

Primer	Nucleotide sequence (5′—3′)
OXA51-F	TAA TGC TTT GAT CGG CCT TG
OXA51-R	TGG ATT GCA CTT CAT CTT GG
OXA23-F	GAT CGG ATT GGA GAA CCA GA
OXA23-R	ATT TCT GAC CGC ATT TCC AT
OXA24-F	GGT TAG TTG GCC CCC TTA AA
OXA24-R	AGT TGA GCG AAA AGG GGA TT
OXA58-F	AAG TAT TGG GGC TTG TGC TG
OXA58-R	CCC CTC TGC GCT CTA CAT AC
ISAb1-F	CAC GAA TGC AGA AGT TG
ISAb1-R	CGA CGA ATA CTA TGA CAC

Screening for the presence of IS*Aba1*

A. baumannii strains were assayed for the IS*Aba1* sequence by PCR with primers IS*Aba1*-F and IS*Aba1*-R (Table 1) giving rise to a 549-bp fragment. A single reaction mixture contained: 30 ng of genomic DNA, 10 pM of each primer, 5 μl of reaction buffer, 1.5 μl of 25 mM $MgCl_2$, 0.5 μl of dNTPs and 0.125 μl of go Taq Polymerase (Promega), with a final volume of 25 μl. The amplification conditions were as follows: initial denaturation (95 °C for 5 min) was followed by 35 cycles of amplification. Each cycle consisted of 95 °C for 45 s, 56 °C for 45 s, 72 °C for 3 min and a final extension step at 72 °C for 5 min [27]. Whether IS*Aba1* preceded the OXA carbapenemase genes was determined using PCR experiments by combinations of the IS*Aba1*-F and reverse primers designed for the blaOXA-23-like, blaOXA-24-like, blaOXA-51-like and blaOXA-58-like genes [9].

Results

A total of 108 non-duplicate *A. baumannii* isolates were collected during the study period, mainly recovered from respiratory (52.8%), wound (29.6%) and urinary tract (11.1%) infections. The primary clinical specimens included tracheal aspirate (31.5%), sputum (29.6%), wound swab (22.2%) and urine (11.1%). The clinical characteristics of *A. baumannii*-infected hospitalized patients were analyzed. The age of the patients ranged from 4 to 85 years (median, 45 years); and 72 (66.7%) were males. The majority (66; 61.1%) of isolates were recovered from patients in the ICU, followed by surgical (19; 17.6%) and medical (13; 12%) wards (Table 2).

The antimicrobials tested and the percentages of isolates determined to be resistant are listed in Table 3. Overall, greater than 75% of

Table 2 Demographic and clinical characteristics of study patients.

Characteristic	No. (%)
Median age in years (Range)	45 (4—85)
Male gender (%)	72 (66.7)
Type of infection[a]	
RTI	57 (52.8)
SSI	32 (29.6)
UTI	12 (11.1)
Primary bacteraemia	7 (6.5)
Type of specimen	
Tracheal aspirate	34 (31.5)
Sputum	26 (24.1)
Wound swab	24 (22.2)
Blood	6 (5.6)
Urine	12 (11.1)
Others[b]	6 (5.6)
Ward admission	
Medical	13 (12)
Surgical	19 (17.6)
Pediatrics	10 (9.3)
ICU	66 (61.1)
Prior antibiotic therapy	52 (48.1)

[a] RTI, respiratory tract infection; SSI, skin and soft tissue infection; UTI, urinary tract infection.
[b] Others; 3 ascitic fluid, 2 stool and one CSF samples

all isolates was resistant to extended-spectrum cephalosporins, aminoglycosides and fluoroquinolones. The rates of susceptibility to colistin, MEP, IMP and trimethoprim—sulfamethoxazole were 95.6, 50, 48.1 and 34.3%, respectively.

The *A. baumannii* isolates were investigated for the presence of OXA-type carbapenemases (Table 4). All isolates harbored the naturally occurring $bla_{\text{OXA-51-like}}$ gene. Of 56 carbapenem-resistant isolates, 48 isolates (85.7%) carried $bla_{\text{OXA-23-like}}$,

Table 3 Number and percentages of *A. baumannii* isolates resistant to selected antimicrobial agents.

Antimicrobial agent	*A. baumannii* isolates ($n = 108$) No. (%)
Ceftazidime	92 (85.2)
Cefotaxime	103 (95.4)
Cefepime	97 (89.8)
Piperacillin	88 (81.5)
Imipenem	56 (51.9)
Meropenem	54 (50)
Ciprofloxacin	89 (82.4)
Levofloxacin	93 (86.1)
Amikacine	81 (75)
Tobramycin	86 (79.6)
Gentamycin	88 (81.5)
Trimethoprim—sulfamethoxazole	71 (65.7)
Colistin	5 (4.6)

Table 4 Distribution of OXA-type β-lactamase genes in 56 carbapenem-resistant *A. baumannii* nosocomial isolates.

bla_{oxa} allele	No. (%)
$bla_{\text{oxa-51}}$ only	2 (3.6%)
ISAba1-$bla_{\text{oxa-51}}$	1 (1.8%)
$bla_{\text{oxa-51}}$ / $bla_{\text{oxa-23}}$	8 (14.3%)
$bla_{\text{oxa-51}}$ / *ISAba1*-$bla_{\text{oxa-23}}$	40 (71.4%)
$bla_{\text{oxa-51}}$ / *ISAba1*-$bla_{\text{oxa-24}}$	3 (5.4%)
$bla_{\text{oxa-51}}$ / $bla_{\text{oxa-58}}$	2 (3.6%)

3 isolates (5.4%) carried $bla_{\text{OXA-40-like}}$ and two isolates (3.6%) carried $bla_{\text{OXA-58-like}}$ genes. The IS*Aba1* element was consistently found upstream of the $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-24}}$ genes in 40 (71.4%) and 3 (5.4%) isolates, respectively, while it was detected upstream of $bla_{\text{OXA-51}}$ in only one (1.8%) isolate.

Discussion

A. baumannii has successfully become a significant nosocomial pathogen because of its remarkable ability to acquire antibiotic resistance and to survive in nosocomial environments. In this study, half of the isolates were of respiratory origin. The other isolates were obtained from other sources, including wounds, urine and blood. This can be partly explained by the fact that *A. baumannii* is the second most frequent pathogen causing respiratory tract infections, such as pneumonia, and was thus more frequently detected in respiratory samples [20]. The findings of this study showed that >60% of *A. baumannii* isolates were obtained from hospitalized patients in ICU wards. This finding is in line with previous reports about the role of *A. baumannii* in nosocomial infections among high-risk ICU patients [1,21].

Overall, the resistance rates of *A. baumannii* to cephalosporins, aminoglycosides and fluoroquinolones obtained in this study were higher than those reported in previous studies in Saudi Arabia [4,5]. Carpabenem resistance in this study is of considerable concern because this class of antimicrobial agents was, until recently, considered to be among the most potent against many microorganisms, including *A. baumannii*. In 2 recent Saudi studies, the resistance rates of *A. baumannii* to IMP and MEM were 62% and 67%, respectively [13,14]. In a 4-year Chinese study, carbapenem resistance increased from 15% for IMP and 23% for MEM in 2008 to 90% and 92% in 2011, respectively [22]. In Taiwan, the prevalence of IMP-resistance in 9 years increased from 3% in 2002 to 59% in 2010 [23].

According to the SENTRY program, the resistance to IMP ranged from 32.8% in North America to 51.7% in Latin America [2]. Polymyxins have emerged as alternatives against *A. baumannii*. In this study, colistin resistance had a prevalence of 4.6%. The SENTRY study reported that the resistance rate to polymyxin B ranged from 2% in North America to 0.9% in Europe [2].

In this study, all 108 *A. baumannii* isolates carried the chromosomally encoded $bla_{\text{OXA-51-like}}$ gene. These findings support those of other studies demonstrating that the detection of the $bla_{\text{OXA-51-like}}$ gene can be used as a supplementary tool to identify the organism at the species level, confirmed by additional methods [24,25].

The most frequent enzymatic mechanism of carbapenem resistance in *A. baumannii* is the production of oxacillinases, and several studies have identified a variety of oxacillinases in carbapenem-resistant *A. baumannii* isolates. The $bla_{\text{OXA-23}}$ carbapenemase-producing *A. baumannii* are becoming widespread globally in Europe, South America, and Asia [10,21,26—29]. In this study, $bla_{\text{OXA-23}}$ carbapenemase was detected in 48 (85.7%) of the 56 carbapenem-resistant isolates and the IS*Aba1* element was located upstream of 40 (71.4%) $bla_{\text{OXA-23}}$-producing strains. It is well established that the promoting sequence IS*Aba*1 has to be present to increase oxacillinase expression and, consequently, to lead to the development of resistance to many antimicrobials, creating a serious problem for the selection of therapy [1,24]. Our data are consistent with the findings of previous studies in that the acquisition of *ISAba1*/$bla_{\text{OXA-23}}$ is the main mechanism for carbapenem resistance among *A. baumannii* isolates in Saudi Arabia [13—15].

The $bla_{\text{OXA-24-like}}$ gene has been reported in Portugal, Spain, Poland, Iran, the United States and Asia [11,12,21,23,30]. In Saudi Arabia, the $bla_{\text{OXA-24-like}}$ gene was detected at a rate of 4—45% in *Acinetobacter* isolates [13,14]. It is noteworthy that 3 (5.4%) *A. baumannii* isolates in our study were positive for IS*Aba1*/$bla_{\text{OXA-24-like}}$ genes. Our findings indicate that there are different mechanisms for carbapenem resistance among *A. baumannii* isolates recovered from different Saudi regions. The spread of these genes among isolates deserves further attention.

In this study, the mechanism of carbapenem resistance is not clear in the 12 carbapenem-resistant isolates encoding the $bla_{\text{OXA-51}}$ gene alone (2 isolates), the $bla_{\text{OXA-51}}$/$bla_{\text{OXA-58}}$ combination (2 isolates) and the $bla_{\text{OXA-51}}$/$bla_{\text{OXA-23}}$ combination (8 isolates) as the sole carbapenemase gene determinants that are not associated with the IS*Aba1* element. Further investigations are required to delineate the resistance mechanism in these isolates (such as the acquisition of metallo-β-lactamases; MBLs or other IS*Ab* elements; IS*Aba2*, IS*Aba3* or IS*Aba4*).

This study had some limitations. The first limitation is the small number of *A. baumannii* isolates and the short duration of the study. Second, this was a single center study. Therefore, our findings may not be generalized to other settings. Further molecular-based epidemiological multi-center studies of longer surveillance duration are necessary to better understand the prevalence and distribution of the carpabenemase genes and prevent the spread of carbapenem-resistant *A. baumannii* nosocomial isolates. These studies are necessary to help determine national priorities for local intervention efforts.

In conclusion, our findings further illustrate the challenge of increasing carbapenem-resistance in *A. baumannii* isolates in Saudi Arabia. The high distribution of class D carbapenemase-encoding genes, mainly IS*Aba1*/OXA-23 and IS*Aba1*/OXA-24 carbapenemases, presents an emerging threat in our hospital. The diversity of resistance genes is particularly worrisome due to the difficult choice of empirical antibiotic therapy in seriously ill patients and the possible contribution to increased hospital stay and associated costs. Moreover, local molecular surveillance is essential to help control carbapenem-resistant *A. baumannii* nosocomial infections and to prevent DNA exchange among endemic nosocomial pathogens.

Conflict of interest

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