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

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KEYWORDS
Acinetobacter baumannii; Carbapenemases; Antibiotics; PCR

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 (ISAba1), 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 blaOXA genes.

Abbreviations: OXA-carbapenemases, carbapenem hydrolyzing class D β-lactamases; ISAba1, insertion sequence.
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Introduction

A. 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 ISaba1, 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 blaOXA-23-carrying *A. baumannii* strains have become endemic [13–15]. Conversely, reports concerning the blaOXA-24-like and blaOXA-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, ISaba1, among *A. baumannii* nosocomial isolates from a Saudi tertiary care hospital over a 6-month period.

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 blaOXA-51-like gene. Of the 56 carbapenem-resistant isolates, 48 isolates (85.7%) carried blaOXA-23-like, 3 isolates (5.4%) carried blaOXA-40-like and two isolates (3.6%) had blaOXA-58-like genes. The ISaba1 element was found upstream of the blaOXA-23 and blaOXA-24 genes in 40 (71.4%) and 3 (5.4%) isolates, respectively, while it was detected upstream of blaOXA-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 ISaba1/OXA-23 and ISaba1/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.

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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, cefazidime, 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 oxacillinas genes

All isolates were subjected to multiplex PCR to detect blaOXA-51-like, blaOXA-23-like, blaOXA-40-like and blaOXA-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 MgCl₂, 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 oxacillinas in A. baumannii isolates [9,19].

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

Screening for the presence of ISAba1

A. baumannii strains were assayed for the ISAba1 sequence by PCR with primers ISAba1-F and ISAba1-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 MgCl₂, 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 ISAba1 preceded the OXA carbapenemase genes was determined using PCR experiments by combinations of the ISAba1-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 percent-ages 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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age in years (Range)</td>
<td>45 (4–85)</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>72 (66.7)</td>
</tr>
<tr>
<td>Type of infection&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>RTI</td>
<td>57 (52.8)</td>
</tr>
<tr>
<td>SSI</td>
<td>32 (29.6)</td>
</tr>
<tr>
<td>UTI</td>
<td>12 (11.1)</td>
</tr>
<tr>
<td>Primary bacteraemia</td>
<td>7 (6.5)</td>
</tr>
<tr>
<td>Type of specimen</td>
<td></td>
</tr>
<tr>
<td>Tracheal aspirate</td>
<td>34 (31.5)</td>
</tr>
<tr>
<td>Sputum</td>
<td>26 (24.1)</td>
</tr>
<tr>
<td>Wound swab</td>
<td>24 (22.2)</td>
</tr>
<tr>
<td>Blood</td>
<td>6 (5.6)</td>
</tr>
<tr>
<td>Urine</td>
<td>12 (11.1)</td>
</tr>
<tr>
<td>Others&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6 (5.6)</td>
</tr>
<tr>
<td>Ward admission</td>
<td></td>
</tr>
<tr>
<td>Medical</td>
<td>13 (12)</td>
</tr>
<tr>
<td>Surgical</td>
<td>19 (17.6)</td>
</tr>
<tr>
<td>Pediatrics</td>
<td>10 (9.3)</td>
</tr>
<tr>
<td>ICU</td>
<td>66 (61.1)</td>
</tr>
<tr>
<td>Prior antibiotic therapy</td>
<td>52 (48.1)</td>
</tr>
</tbody>
</table>

<sup>a</sup> RTI, respiratory tract infection; SSI, skin and soft tissue infection; UTI, urinary tract infection.
<sup>b</sup> 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 blaoxa-51-like gene. Of 56 carbapenem-resistant isolates, 48 isolates (85.7%) carried blaoxa-23-like,

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

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>A. baumannii isolates (n = 108)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>92 (85.2)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>103 (95.4)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>97 (89.8)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>88 (81.5)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>56 (51.9)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>54 (50)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>89 (82.4)</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>93 (86.1)</td>
</tr>
<tr>
<td>Amikacine</td>
<td>81 (75)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>86 (79.6)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>88 (81.5)</td>
</tr>
<tr>
<td>Trimethoprim—sulfamethoxazole</td>
<td>71 (65.7)</td>
</tr>
<tr>
<td>Colistin</td>
<td>5 (4.6)</td>
</tr>
</tbody>
</table>

3 isolates (5.4%) carried blaoxa-40-like and two isolates (3.6%) carried blaoxa-58-like genes. The ISAba1 element was consistently found upstream of the blaoxa-23 and blaoxa-24 genes in 40 (71.4%) and 3 (5.4%) isolates, respectively, while it was detected upstream of blaoxa-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]. Carbapenem 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].

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

<table>
<thead>
<tr>
<th>blaoxa allele</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaoxa-51 only</td>
<td>2 (3.6%)</td>
</tr>
<tr>
<td>ISAba1-blaoxa-51</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>blaoxa-51/blaoxa-23</td>
<td>8 (14.3%)</td>
</tr>
<tr>
<td>blaoxa-51/ISAba1-blaoxa-23</td>
<td>40 (71.4%)</td>
</tr>
<tr>
<td>blaoxa-51/ISAba1-blaoxa-24</td>
<td>3 (5.4%)</td>
</tr>
<tr>
<td>blaoxa-51/blaoxa-58</td>
<td>2 (3.6%)</td>
</tr>
</tbody>
</table>
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 blaOXA-51-like gene. These findings support those of other studies demonstrating that the detection of the blaOXA-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 blaOXA-23 carbapenemase-producing A. baumannii are becoming widespread globally in Europe, South America, and Asia [10,21,26–29]. In this study, blaOXA-23 carbapenemase was detected in 48 (85.7%) of the 56 carbapenem-resistant isolates and the ISAb1 element was located upstream of 40 (71.4%) blaOXA-23-producing strains. It is well established that the promoting sequence ISAb1 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 ISAb1/blaOXA-23 is the main mechanism for carbapenem resistance among A. baumannii isolates in Saudi Arabia [13–15].

The blaOXA-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 blaOXA-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 ISAb1/blaOXA-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 blaOXA-51 gene alone (2 isolates), the blaOXA-51/blaOXA-58 combination (2 isolates) and the blaOXA-51/blaOXA-23 combination (8 isolates) as the sole carbapenemase gene determinants that are not associated with the ISAb1 element. Further investigations are required to delineate the resistance mechanism in these isolates (such as the acquisition of metallo-β-lactamases; MBLs or other ISAb elements; ISAb2, ISAb3 or ISAb4).

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 carbapenemase 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 ISAb1/OXA-23 and ISAb1/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

Funding: No funding sources.

Competing interests: None declared.

Ethical approval: Not required.

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