Clonal diversity of *Acinetobacter baumannii* from diabetic patients in Saudi Arabian hospitals

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Carbapenem-resistant *Acinetobacter baumannii* (CR-AB) represents a major health-care problem, causing high rates of morbidity and mortality. This study investigated the clonality of CR-AB isolated from diabetic patients from different regions in Saudi Arabia, as well as the relatedness of the β-lactamase genes. A total of 64 non-repetitive CR-AB clinical isolates were collected from 16 different regions in Saudi Arabia from intensive care patients. Isolates were identified phenotypically by the Vitek 2 compact system and genotypically by amplification of the *bla*OXA-51-like* gene. The target sequences were amplified by PCR and the clonal diversity of the isolates was explored by PFGE. Resistance studies revealed that the prevalence of imipenem and meropenem resistance was 92 % and 96 %, respectively, while the vast majority of the isolates were susceptible to tigecycline and colistin. In addition, *bla*VIM and *bla*OXA-23 were the most prevalent genes in the isolates under investigation, while IS\textsubscript{Aba1} was the most dominant insertion sequence. PFGE results showed 13 clusters; clone H was dominant, comprising 20 isolates from four hospitals, followed by clones C and F, comprising 11 isolates each from three and six hospitals, respectively. Moreover, the current study signified the clonal diversity of CR-AB in Saudi Arabia and showed the ability of some clones to infect patients in many different cities.

INTRODUCTION

*Acinetobacter baumannii* is an aerobic, non-motile, non-fermenting, Gram-negative, opportunistic pathogen that plays a major role in nosocomial infections of immunocompromised patients (Brown & Amyes, 2006). It is considered one of the six most important multidrug-resistant micro-organisms in hospitals, especially in intensive care units (ICUs). Infections with this pathogen are often associated with high rates of morbidity and mortality (Papp-Wallace et al., 2011). *A. baumannii* intrinsically has low susceptibility to different antimicrobial agents (Lee et al., 2011). In the last decade, many multi-drug-resistant (MDR) and extensive-drug-resistant *A. baumannii* have been isolated globally (Souli et al., 2008), regionally (Alsweih et al., 2012; Shahcheraghi et al., 2011) and locally (Abdalhamid et al., 2014; Alsultan et al., 2013). Nowadays, MDR *A. baumannii* are among the most difficult pathogens to treat (Maragakis & Perl 2008). Carbapenems represent the main therapy for serious infections caused by such pathogens (Mohajeri et al., 2013, Qi et al., 2008). Unfortunately, a dramatic increase in carbapenem-resistant *A. baumannii* (CR-AB) isolates has been recorded in recent years (Alsultan et al., 2013; Shahcheraghi et al., 2011).

(class D) mainly mediate resistance to carbapenems, and to a lesser extent class A, e.g. KPC (Brown & Amyes, 2006). Since the discovery of the first OXA-type carbapenemases in 1993 in an A. baumannii isolate from Scotland (Paton et al., 1993), many types have been discovered and the number of OXA-type β-lactamases is exponentially increasing (Lee et al., 2011). Five main groups of OXA carbapenemases are involved in the resistance of A. baumannii: OXA-23-like, OXA-40-like, OXA-51-like, OXA-58-like and OXA-143 enzymes (Brown & Amyes, 2006). The genes coding these enzymes are regulated by upstream insertion sequences (IS), specifically ISAb1, ISAb2, ISAb3 and IS18 (Brown & Amyes, 2006; Heritier et al., 2006; Peleg et al., 2008). These insertion elements play a major role in the expression of such genes (Poirel & Nordmann 2006b; Turton et al., 2006a). Chromosomally located blaOXA-51-like genes are intrinsically present in all A. baumannii stains (Turton et al., 2006b). In addition to OXA carbapenemases, three MBLs have been detected in A. baumannii, namely IMP, VIM and SIM types (Peleg et al., 2008). In Saudi Arabia, the data regarding the mechanism of carbapenem resistance in A. baumannii are limited.

The aim of the present study was to investigate the prevalence of the genes and different insertion sequences associated with carbapenem resistance in A. baumannii clinical isolates from 16 different regions in Saudi Arabia. In addition, the clonal relatedness of such clinical isolates was determined.

**METHODS**

**Bacterial isolates.** Sixty-four non-repetitive A. baumannii clinical isolates were collected from clinical specimens from diabetic patients in ICUs. The isolates were collected from 16 different regions in Saudi Arabia between January and November 2012. No ethical approval was required because samples were collected as a routine part of standard patient care. Conventional microbiological methods were performed for preliminary identification of the isolates, and the identification was confirmed by the Vitek 2 compact system (bioMerieux) according to the guidelines of the manufacturer. For molecular confirmation of the Vitek 2 compact system identification, PCR was used to detect the intrinsic blaOXA-51-like gene. The primers used for amplification of this gene are depicted in Table 1.

**Antimicrobial susceptibility testing.** The resistance profile of the isolates against imipenem, meropenem, tigecycline and colistin was initially determined by the Vitek 2 compact system. It was subsequently confirmed by agar diffusion assay according to the Clinical and Laboratory Standards Institute (CLSI, 2012) using antibiotic discs (Oxoid). Minimum inhibitory concentrations (MICs) were determined by Etest strips (AB Biodisk) according to the manufacturer’s instructions. All experiments were carried out in triplicate.

**Detection of blaOXA-23 and blaOXA-40 genes.** PCR was used to amplify the genes encoding OXA-type carbapenemases (blaOXA-23, blaOXA-40) and MBLs (blaVIM, blaIM, blaSIM, blaIMP and blaSPM) as previously described (Ellington et al., 2007; Poirel & Nordmann 2006b; Qi et al., 2008; Woodford et al., 2006), using the primers depicted in Table 1.

**Molecular typing by PFGE.** Sixty-four A. baumannii clinical isolates were typed by PFGE analysis as previously described (Miranda et al., 2007; Poirel & Nordmann 2006).

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**Table 1. Nucleotide sequence of primers used in this study**

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Nucleotide sequence (5′→3′)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>OXA-51-F</td>
<td>TAATGCTTTTGATCGGCCCTTG</td>
<td>Woodford et al. (2006)</td>
</tr>
<tr>
<td>OXA-51-R</td>
<td>TGGATTGACCTCATCTTGG</td>
<td>Woodford et al. (2006)</td>
</tr>
<tr>
<td>OXA-23-F</td>
<td>GATCGGATTGGAGAACAGA</td>
<td>Qi et al. (2008)</td>
</tr>
<tr>
<td>OXA-23-R</td>
<td>ATTCTTGACCGCATTTCCAT</td>
<td>Qi et al. (2008)</td>
</tr>
<tr>
<td>OXA-40-F</td>
<td>GGTGTTGGGCCCCCTTAAA</td>
<td>Qi et al. (2008)</td>
</tr>
<tr>
<td>OXA-40-R</td>
<td>AGTTGAGCGAAAAAGGGATT</td>
<td>Qi et al. (2008)</td>
</tr>
<tr>
<td>ISAb1-F</td>
<td>GTCCTTTGGCTCATGTCATGC</td>
<td>Poirel &amp; Nordmann (2006b)</td>
</tr>
<tr>
<td>ISAb1-R</td>
<td>CATGTAACCAATGCTCACC</td>
<td>Poirel &amp; Nordmann (2006b)</td>
</tr>
<tr>
<td>ISAb2-F</td>
<td>AATCCGAGATAGACGGGTTC</td>
<td>Poirel &amp; Nordmann (2006b)</td>
</tr>
<tr>
<td>ISAb2-R</td>
<td>TGACACATAACCTAGTCAC</td>
<td>Poirel &amp; Nordmann (2006b)</td>
</tr>
<tr>
<td>ISAb3-F</td>
<td>CAACTAAATGTCACCTCCG</td>
<td>Poirel &amp; Nordmann (2006b)</td>
</tr>
<tr>
<td>ISAb3-R</td>
<td>CGTTTACCCCAAAAATAGGC</td>
<td>Poirel &amp; Nordmann (2006b)</td>
</tr>
<tr>
<td>IS18-F</td>
<td>CACCCAACTTTTCAGATCG</td>
<td>Poirel &amp; Nordmann (2006b)</td>
</tr>
<tr>
<td>IS18-R</td>
<td>ACCAGCCATAACTCTACTCG</td>
<td>Poirel &amp; Nordmann (2006b)</td>
</tr>
<tr>
<td>IMP-F</td>
<td>GGATAGATGGTGGCTATTYCTTC</td>
<td>Ellington et al. (2007)</td>
</tr>
<tr>
<td>IMP-R</td>
<td>CCAAACTACAGTTATCT</td>
<td>Ellington et al. (2007)</td>
</tr>
<tr>
<td>VIM-F</td>
<td>GATGTTGTGTGGTCCATA</td>
<td>Ellington et al. (2007)</td>
</tr>
<tr>
<td>VIM-R</td>
<td>CGAATGCAGCAGCAAGC</td>
<td>Ellington et al. (2007)</td>
</tr>
<tr>
<td>GIM-F</td>
<td>TCGACACACCCTTGTCGAA</td>
<td>Ellington et al. (2007)</td>
</tr>
<tr>
<td>GIM-R</td>
<td>AACTCTACACCTTGCCATGC</td>
<td>Ellington et al. (2007)</td>
</tr>
<tr>
<td>SPM-F</td>
<td>AAAATCGGTCGACGCAACG</td>
<td>Ellington et al. (2007)</td>
</tr>
<tr>
<td>SPM-R</td>
<td>ACATTATCGCGTGGCAACAG</td>
<td>Ellington et al. (2007)</td>
</tr>
<tr>
<td>SIM-F</td>
<td>TACAAGGCGATGGCCATCG</td>
<td>Ellington et al. (2007)</td>
</tr>
<tr>
<td>SIM-R</td>
<td>TAATGCGCTTTCCCATG</td>
<td>Ellington et al. (2007)</td>
</tr>
</tbody>
</table>
RESULTS

Clinical isolates and antimicrobial susceptibility

Sixty-four non-repetitive *A. baumannii* clinical isolates (coded DM001 to DM064) were collected from 16 different regions in Saudi Arabia. The isolates were cultured from different clinical specimens of diabetic patients admitted to ICUs. Thirty-four patients (53 %) were male while female patients represented 47 % (30/64). Most isolates were collected from respiratory tract clinical specimens (41 out of 64, 64 %), while the remainder were obtained from infected blood, urine, abdomen and skin. The Vitek 2 compact system was used to identify the isolates, and the identification was confirmed after amplification of the genetic targets coding two OXA b-lactamases and insertion sequences.

All isolates showed resistance to at least one of the two tested carbapenems (imipenem and meropenem), as shown in Fig. 1. There was no significant difference between the prevalence of imipenem (59 out of 64, 92 %) and meropenem (62 out of 64, 96 %) resistance among the tested isolates, as shown in Table 2. On the other hand, the majority of the isolates showed susceptibility to tigecycline and colistin, 97 % (Table 2).

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Number of isolates (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive (S)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>3 (4.7)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>62 (96.9)</td>
</tr>
<tr>
<td>Colistin</td>
<td>62 (96.9)</td>
</tr>
</tbody>
</table>

DISCUSSION

The emergence and global distribution of CR-AB represent a major problem in the health care setting, especially in ICUs. *A. baumannii* is a notorious opportunistic pathogen mainly associated with hospital-acquired infections (Wang et al., 2013). This pathogen causes serious hospital-acquired infections associated with a high mortality rate, particularly in immunocompromised patients. Moreover, very limited therapeutic options (e.g. colistin and tigecycline) are available for treatment of infection caused by such pathogens (Bassetti et al., 2011).

The recent worldwide emergence of carbapenem resistance in Gram-negative bacteria has mainly been manifested by the infiltration of different types of b-lactamase. Carbapenemases were the drug of choice for treating infections caused by MDR *A. baumannii*; however, resistance to such antimicrobial agents is now a common occurrence and...
pan-drug resistant strains are beginning to emerge (Brown & Amyes, 2006). In A. baumannii, the main carbapenems hydrolysing β-lactamases are OXA-type carbapenemases (Ambler class D β-lactamases) and MBLs (class B β-lactamases) (Poirel et al., 2012).

In the present study, 64 A. baumannii clinical isolates (based on the presence of blaoXA-51-like gene) were collected from tertiary care hospitals located in 16 different cities in Saudi Arabia. These cities were distributed in many different provinces separated by large geographical distances. The majority of the isolates were recovered from respiratory tract secretions. The vast majority of the isolates were susceptible to colistin and tigecycline (97 %). A higher tigecycline resistance rate (9.7 %) was recently recorded in Saudi Arabia. These cities were distributed in many different provinces separated by large geographical distances. The majority of the isolates were recovered from respiratory tract secretions.

The prevalence of OXA-23 in the current study was 53.1 %, which is comparable to recently published results from Egypt (50 %) (Al-Agamy et al., 2013) and India (47.9 %) (Karunasagar et al., 2013). A higher prevalence of OXA-23 was detected in CR-AB isolates from Riyadh (Aly et al., 2014), the eastern region of Saudi Arabia (80.4 %) (Abdalhamid et al., 2014) and Iran (84 % (Shahcheraghi et al., 2011) and 77.9 % (Mohajeri et al., 2013)). In contrast, only two out of 40 (5 %) isolates collected from Kuwait carried blaoXA-23 (Al-Sweih et al., 2012), while only one isolate out of 92 (1.1 %) isolated from Taiwan harboured this gene (Lu et al., 2009). On the other hand, blaoXA-40 was amplified from 29.7 % of our isolates. This carbapenemase was not detected in any of the 253 tested isolates from Riyadh (Aly et al., 2014). While blaoXA-40 was the most prevalent acquired gene (57.6 %) in 59 isolates from Spain, none of those isolates harboured blaoXA-23 (Villalón et al., 2013). In addition, 7.5 %, 19.2 % and 22.9 % of isolates from Egypt (Al-Agamy et al., 2014), Iran (Mohajeri et al., 2013) and India (Karunasagar et al., 2011), respectively, contained the OXA-40 β-lactamase. Co-existence of both blaoXA-23 and blaoXA-40 was not detected in any of our isolates. In contrast, 45 % and 16.4 % of CR-AB isolates collected from Egypt (Al-Agamy et al., 2014) and Iran (Mohajeri et al., 2013), respectively, co-produced both the OXA-23 and OXA-40 β-lactamases.

Beside OXA carbapenemases, MBLs were detected in many of the isolates. VIM and SPM β-lactamases were the only two MBLs detected, where their prevalence was 92.2 % (59/64) and 28.1 % (18/64), respectively, while the other three MBLs (IMP, GIM and SIM) were absent. A lower prevalence of MBLs (6 %) has been recorded in Iran (Shahcheraghi et al., 2011), where only blaspmp was detected while blavim was completely absent. In addition, 46 CR-AB clinical isolates from the eastern region of Saudi Arabia harboured neither blavim nor blaimp (Abdalhamid et al., 2014). Moreover, MBL encoding genes were not amplified from any of 40 clinical isolates from Egypt (Al-Agamy et al., 2014). In contrast, 72.5 % (29/40) of CR-AB isolates from Kuwait carried blagenes encoding VIM and IMP MBLs (Al-Sweih et al., 2012).

Our results show that ISAba1 was the most prominent (90.6 %) insertion element, followed by ISAba3 (20.3 %), while ISAba2 was detected in only six isolates out of 64 (9.4 %). The prevalence of ISAba1 is comparable to that

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of isolates (n, %)</th>
</tr>
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<tbody>
<tr>
<td>blaoXA-23</td>
<td>34 (53.1)</td>
</tr>
<tr>
<td>blaoXA-40</td>
<td>19 (29.7)</td>
</tr>
<tr>
<td>Co-existence of both blaoXA-23 and blaoXA-40</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Absence of both blaoXA-23 and blaoXA-23</td>
<td>0 (0)</td>
</tr>
<tr>
<td>blavim</td>
<td>59 (92.2)</td>
</tr>
<tr>
<td>blaspmp</td>
<td>18 (28.1)</td>
</tr>
<tr>
<td>Co-existence of both blavim and blaspmp</td>
<td>17 (26.6)</td>
</tr>
<tr>
<td>blaimp</td>
<td>0 (0)</td>
</tr>
<tr>
<td>blag2em</td>
<td>0 (0)</td>
</tr>
<tr>
<td>blag4em</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ISaba1</td>
<td>58 (90.6)</td>
</tr>
<tr>
<td>ISaba2</td>
<td>6 (9.4)</td>
</tr>
<tr>
<td>ISaba3</td>
<td>13 (20.3)</td>
</tr>
<tr>
<td>IS18</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Co-existence of both ISaba1 and ISaba2</td>
<td>5 (7.8)</td>
</tr>
<tr>
<td>Co-existence of both ISaba1 and ISaba3</td>
<td>11 (17.2)</td>
</tr>
</tbody>
</table>
recorded in 59 isolates from Spain (93.2 %) (Villalón et al., 2013). A lower prevalence was recorded in Taiwan (36 %) (Lu et al., 2009) and India (33 %) (Karunasagar et al., 2011). The presence of different insertion sequences renders \textit{A. baumannii} resistant to carbapenems (Lee et al., 2011). Such insertion sequences are located in the proximity of genes coding different OXA-type carbapenemases and involved in their overexpression (Corvec et al., 2003).

In the current work, the genetic similarity of PFGE types was very high (89 to 100 %). The clonal diversity revealed two types of epidemic clone: monoclonal and polyclonal. The monoclonal model showed that the most common clones appeared in 12 of 16 cities. These monoclonal outbreaks were caused by one or more epidemic PFGE type. The polyclonal model has affected four cities (Riyadh, Almadinah, Tabok and Kamis-Mosaiti). Riyadh is the capital city of Saudi Arabia, and patients harboured nine out of the 13 different clones, while Almadinah, the capital of Almadinah province, had five different clones. Both cities could experience explosive outbreaks at any time. Clone F has been detected in hospitals in Riyadh, Almadinah, Tabok, Abha, Alrass and Alkafi. This finding suggests transmission of clones not only from one hospital to another, but also between distant health regions. The promiscuity of \textit{A. baumannii} transmission has been recognized (Coelho et al., 2006; Dijkshoorn et al., 1996; Manikal et al., 2000), suggesting that the reappearance of certain clones within these hospitals reflects the endemic persistence of this pathogen in diabetic patients, hospitals and environments that may represent a risk factor for future outbreaks.

In conclusion, the results of the current study reveal that OXA-23 and VIM were the most common \(\beta\)-lactamases conferring carbapenem resistance to \textit{A. baumannii}. In addition, IS\textit{Aba}1 was the most prevalent insertion sequence. Moreover, the current study not only signifies the clonal diversity of CR-AB in Saudi Arabia, but also the ability of certain clones to infect patients in many different cities.

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