Characterization of carbapenem-resistant
Acinetobacter baumannii clinical isolates in a tertiary care hospital in Saudi Arabia

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SUMMARY

This study characterized the occurrence of carbapenem resistance of Acinetobacter baumannii isolates in a tertiary care hospital in Saudi Arabia. From January 2010 until February 2012, Acinetobacter spp. isolates were collected from different wards and were identified using Vitek 2 system and 16S rRNA gene sequencing. Vitek 2 system and Etest were used for susceptibility testing. PCR and Pulse field gel electrophoresis (PFGE) were used for detecting and typing genes associated with carbapenem resistance. A total of 141 isolates were identified as A. baumannii. A total of 46 (32.6%) isolates were carbapenem-resistant Acinetobacter baumannii (CRAB) isolates and had wild diversity by PFGE. Metallo β-lactamase confirmatory test was positive for 43 isolates with negative PCR for blaIMP and blavim. Among the 46 CRAB strains, 37 isolates harbored blaOXA-23 which was encoded downstream of ISAbal and 1 isolate had ISAbal encoded upstream blaOXA-23. These data reveal that the interhospital transmission of CRAB isolates was apparently insignificant. BlaOXA-23 adjacent to ISAbal was the main mechanism of carbapenem resistance in these isolates. To our knowledge, this is the first molecular study characterizing carbapenem resistance in A. baumannii in the Eastern Province of Saudi Arabia.

KEY WORDS: Acinetobacter baumannii, Carbapenem resistance.

INTRODUCTION

Acinetobacter baumannii is an emerging opportunistic organism causing a wide variety of nosocomial infections such as wound infections, ventilator-associated pneumonia, bloodstream infections, intensive care unit (ICU) infections, and urinary tract infections (Maragakis et al., 2008; Peleg et al., 2008; Poirel et al., 2006; Tognim et al., 2004). The treatment of A. baumannii is difficult because of the emergence of antimicrobial resistance (Maragakis et al., 2008; Peleg et al., 2008; Poirel et al., 2006; Tognim et al., 2004). There is no single agent against the organism, requiring a combination therapy (Al-Tawfiq et al., 2007). Multi-drug resistant A. baumannii, defined as an A. baumannii strain resistant to at least three different groups of antibiotics, has emerged and is reported worldwide to significantly increase the morbidity, mortality, and cost of such infections (Maragakis et al., 2008; Peleg et al., 2008; Poirel et al., 2006; Tognim et al., 2004). The identification of antimicrobial resistance mechanisms in A. baumannii will also improve the outcome of infections caused by this organism. A. baumannii resistance to carbapenem has been documented worldwide in the last few years (Al Johani et al., 2010; Al-Sweih et al., 2012; Andriamanantena et al., 2010; Boo et al., 2009; Dijkshoorn et al., 2005; Giannouli et al., 2010; Gur et al., 2008; Khan et al., 2012; Nemec et al., 2008;
Stoeva et al., 2008). The mechanisms of carbapenem resistance in A. baumannii include changes in penicillin-binding protein, alterations in outer membrane proteins, or the production of carbapenemases (Dijkshoorn et al., 2005; Peleg et al., 2008; Stoeva et al., 2008). Carbapenemases, oxacillinases (OXA) or metallo-β-lactamases (MBLs) are of major concern because of their ability for rapid dissemination. Although MBLs, IMP-β-lactamases and VIM-β-lactamases have been described in A. baumannii, the most widespread β-lactamasmes with carbapenem activity in A. baumannii are oxacillinases. OXA enzymes, encoded by blaOXA genes, can be classified into eight distinct subgroups, of which OXA-23-like, OXA-24-like, OXA-51-like, and OXA-58-like have been identified in A. baumannii (Maragakis et al., 2008; Peleg et al., 2008; Poirel et al., 2006). Several reports have revealed that blaOXA-51 gene is intrinsic to A. baumannii which can be used as a marker for detection of A. baumannii (Brown et al., 2005a; Brown et al., 2005b; Heritier et al., 2005; Segal et al., 2005; Turton et al., 2006b; Wroblewska et al., 2007). There is a cumulative body of data suggesting that blaOXA-23, blaOXA-27, and blaOXA-51 are regulated in part by insertion sequences located upstream of these genes resulting in gene overexpression and carbapenem resistance (Brown et al., 2005; Segal et al., 2005; Turton et al., 2006a; Turton et al., 2006b). A potential candidate is the ISAba1 element, belonging to the IS4 family, and has been detected upstream of blaOXA-23, blaOXA-27, and blaOXA-51 (Nemec et al., 2008; Segal et al., 2005; Turton et al., 2006a).

The aim of this study was to molecularly characterize carbapenem resistance (CR) in multidrug-resistant (MDR) Acinetobacter baumannii strains isolated from different wards in the King Fahad Specialist Hospital-Dammam (KFSHD), Eastern Province, Saudi Arabia. Acinetobacter baumannii isolates were detected using the Vitek 2 system and ribosomal DNA sequencing methods. Based on the minimum inhibitory concentration (MIC) profiles, carbapenem-resistant Acinetobacter baumannii strains were screened using metallo-beta-lactamase (MBL) Etest and multiplex PCR using primers designed to detect the most common carbapenemase gene families encoding OXA β-lactamases and MBLs.

**METHODOLOGY**

**Clinical strains and organism identification**

From January 2010 until February 2012, a total of 141 non-repetitive clinical isolates of Acinetobacter spp. were collected from the hospital. Bacterial strains were obtained from the intensive care unit (66/141, 46.8%) and other hospital wards (75/141, 53.2%) and were primarily identified using conventional methods. All clinical strains of Acinetobacter spp. were evaluated using the Vitek 2 automatic system (Biomerieux, France) for full identification using the identification card for Gram negative strains (ID-GNB) according to the manufacturer’s recommendations. The Microseq 500 16S rDNA bacterial identification kit (Applied Biosystem, Foster City, CA, USA) was used to identify isolates to species level.

**Antimicrobial susceptibility test and MBL etest**

Susceptibility of the clinical isolates to antimicrobial agents was tested using the Vitek 2 system. All results were interpreted using the advanced expert system (AES) (software version VT2-R04.03). MICs and breakpoints were determined according to Clinical and Laboratory Standards Institute (CLSI) recommendations. In addition, tigecycline MICs were interpreted according to the FDA breakpoints. E. coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as controls for the antimicrobial susceptibility testing. Susceptibility of A. baumannii isolates to meropenem and imipenem was confirmed by determination of MICs with Etest strips (Biomerieux, France). To detect the presence of metallo-β-lactamase (MBL)-producing Acinetobacter baumannii, MBL E-test strips with imipenem (IMP) and imipenem plus EDTA (IMPI) were used according to the manufacturer’s recommendations. Carbapenem-resistant A. baumannii (CRAB) isolates were further analyzed using molecular biology tools.

**Pulse field gel electrophoresis**

CRAB strains were genotyped and classified into genetic clusters using pulsed-field gel electrophoresis (PFGE) for ApaI-digested total chromosomal DNA prepared as described pre-
Carbapenem Resistant Acinetobacter baumannii strains

previously (Bannerman et al., 1995) in a CHEF-DR II apparatus (Bio-Rad Laboratories, Richmond, CA, USA). The following parameters were used: 200 V for 22 hours at 14°C, with a ramped pulse 5-13 seconds. Gels were stained with ethidium bromide, destained 2-3 times in distilled water, viewed under ultraviolet light, photographed, and analyzed using a Quantity One Gel Doc software photograph. The clonal dissemination index (CDI) was estimated by determining the ratio of infected patients to clones.

**PCR analysis and sequencing of carbapenemase genes**

The 46 CRAB isolates were further analyzed for carbapenemase genes using the multiplex PCR for the following genes IMP-, VIM-, and OXA-carbapenemases (OXA23, OXA24, OXA51, and OXA58). The PCR assay was performed according to Grobner et al. (Grobner et al., 2009). In addition, a PCR assay was performed to detect NDM1 gene as described by Chihara et al. (Chihara et al., 2011). A PCR using primers for bla\textsubscript{OXA-51} was performed to identify bla\textsubscript{OXA-51} in A. baumannii clinical isolates, ATCC BAA 747 strain, and in 6 previously identified clinical isolates of A. lwofii and a clinical isolate of A. junii.

DNA extracts used as positive controls for bla\textsubscript{IMP}, bla\textsubscript{VIM}, bla\textsubscript{NDM1}, bla\textsubscript{OXA-23}, bla\textsubscript{OXA-24}, bla\textsubscript{OXA-51}, and bla\textsubscript{OXA-58} were kindly provided by Dr. Nancy Hanson, Department of Microbiology and Immunology, Creighton University, Omaha, NE, USA and by Dr. Yvonne Pfeifer, Robert Koch Institute, Wernigerode, Germany. Molecular grade water control was used as a negative control in PCR assays to detect contamination. The full length gene of bla\textsubscript{OXA-23} was amplified by PCR using OXA23FLF and OXA23FLR primers as previously described (Mugnier et al., 2010) and was sequenced using the ABI 3730xl DNA analyzer (Applied Biosystems, Foster city, CA, USA). The IS\textsubscript{Aba1} element was identified using the IS\textsubscript{Aba1}F (CACGAATGCAGAAGTTG) and

<table>
<thead>
<tr>
<th>Reference</th>
<th>Amplicon Size</th>
<th>Sequence</th>
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</tr>
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<tr>
<td>OXA24F</td>
<td>GGTTAGTTGGCCCCCTTTAAA</td>
<td>246</td>
<td>Woodford et al., 2006</td>
</tr>
<tr>
<td>OXA24R</td>
<td>AGTTGAGCGAAAAAGGGGATT</td>
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<td></td>
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<tr>
<td>OXA51F</td>
<td>TAATGCTTTGATCGGCTTGG</td>
<td>353</td>
<td></td>
</tr>
<tr>
<td>OXA51R</td>
<td>TGGATTGCACCTCATTGG</td>
<td></td>
<td></td>
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<tr>
<td>OXA58F</td>
<td>AAGATTTGGGGGCTTTGTGCTG</td>
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<td>CCCCTCTGCGCTCTACATAC</td>
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<td></td>
</tr>
<tr>
<td>ImpF</td>
<td>GAATAGAATGTGTTAACTCTTC</td>
<td>188</td>
<td>Mendes et al., 2007</td>
</tr>
<tr>
<td>ImpR</td>
<td>CCAAACCACTAGGTATC</td>
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<tr>
<td>VIMF</td>
<td>GTTTGTCGCATACGCAAC</td>
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<td>VIMR</td>
<td>AATGCAGCGACCCAGATG</td>
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<tr>
<td>OXA23FLF</td>
<td>GGATTCCATGAAATATTATTACTTCG</td>
<td>822</td>
<td>Mugnier et al., 2010</td>
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<td>OXA23FLR</td>
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<td>GAGATTGCAGACGACTTTG</td>
<td>497</td>
<td>Chihara et al., 2011</td>
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<td>NDM1R</td>
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OXA23R primers and the ISAba1F and OXA51R primers as described by Turton et al. (Turton et al., 2006a). In addition, a PCR using ISAba1F and ISAba1R (CGACGAATCATATGCCAC) primers (Turton et al., 2006a) were conducted on a total of 5 representative carbapenem-susceptible A. baumannii strains to detect the ISAba1 element. Primers used in this study as described in the literature (Mendes et al., 2007; Mugnier et al., 2010; Chihara et al., 2011; Woodford et al., 2006) are listed in Table 1.

RESULTS

Bacterial strain identification and antimicrobial susceptibility testing
All 141 clinical isolates identified as Acinetobacter baumannii complex using Vitek 2 were identified as Acinetobacter baumannii using Microseq 500 16S rDNA bacterial identification kit. All A. baumannii strains were susceptible to colistin. The percentage of CRAB isolates was 32.6% (n=46). The highest rates of susceptibility was observed for amikacin (73%, n=103) and for tigecycline (70.9%, n=100). The susceptibility rates were 45.4% (n=64) for gentamicin, 36.9% (n=52) for cotrimoxazole, 26.2% (n=37) for cefepime, 14.9% (n=21) for ceftazidime, and 30.5% (n=43) for ciprofloxacin. The resistance rates were 32.6% (n=46) for imipenem and 33.3% (n=47) for both meropenem and piperacillin-tazobactam. A total of 43 of CRAB strains were positive using MBL E-test strips.

Epidemiological data
The clinical characteristics associated with the 46 CRAB isolates were analyzed. The age of the patients ranged from 16 to 97 years (median, 46 years); 21 (46%) patients were males and 25 (54%) were females. The hospital-associated infections were 27 (58.7%) and the healthcare associated infections were 12 (26.1%). In addition, the community-acquired infections were 7 (15.2%) with no hospitalization or access to healthcare in the last three months. The majority 26/46 (56%) of the isolates were recovered from patients in the adult intensive care unit (AICU), whose admission diagnoses included hospital associated pneumonia (n=9) and ventilator-associated pneumonia (n=2) among others. The sites of infections were 24/46 (52%) respiratory samples, 12/46 (26%) wound cultures, and 10/46 (22%) urine cultures. Respiratory specimens included endotracheal tube suction (ETT) (n=12), sputum (n=10) and bronchoalveolar lavage (BAL) (n=2).

FIGURE 1 - Pulse-field gel electrophoresis (PFGE) patterns in representative strains of CRAB. Strains were isolated from different hospital wards. Strains 6 and 21 and strains 50 and 69 are indistinguishable whereas the other strains are unrelated.
PFGE revealed that the majority of the isolates had high diversity with multivariate clones. A total of 44 different clones were identified among the 46 isolates. A group of 2 strains was indistinguishable (representative strains 6 and 21, Figure 1) and another unrelated group of 2 strains was indistinguishable (representative strains 50 and 69, Figure 1). The CDI did not significantly exceed 1. These strains were recovered from different patients at different times from different wards.

Molecular analysis of the strains and carbapenemase genes

All 141 clinical strains and ATCC BAA 747 strain harbored a PCR product of 353 bps correlating with \( \text{bla}_{\text{OXA-51}} \) like. None of the Acinetobacter spp. other than \text{baumannii} (6 strains) harbored \( \text{bla}_{\text{OXA-51}} \) like.

In the 46 CRAB isolates, a PCR product of 501 bps correlating with \( \text{bla}_{\text{OXA-23}} \) was detected in 80.4% (n=37) of the isolates and was confirmed to be \( \text{bla}_{\text{OXA-23}} \) by sequencing the full length PCR product (822 bps). All 37 CRAB isolates that were PCR positive for \( \text{bla}_{\text{OXA-23}} \) had an expected band of 1.6 kb in a PCR using ISAba1F and OXA51R. Although all of these isolates were PCR positive for \( \text{bla}_{\text{OXA-51}} \), none gave a band in the PCR using ISAba1F and OXA51R.

The ISAbal element was detected upstream \( \text{bla}_{\text{OXA-51}} \) in only 1 \( \text{bla}_{\text{OXA-23}} \) non-producing CRAB isolates with an expected band of 1.2 kb. There were 8 isolates encoding only \( \text{bla}_{\text{OXA-51}} \) gene and was not associated with the ISAbal element. The ISAbal element was not detected in any of the 5 representative carbapenem susceptible \text{A. baumannii} strains. \( \text{Bla}_{\text{NDM1}}, \text{Bla}_{\text{IMP}} \) or \( \text{Bla}_{\text{VIM}} \) metallo-\( \beta \)-lactamases were not detected in any of the strains.

DISCUSSION

Acinetobacter baumannii has emerged as a threat to hospitalized patients as they can acquire resistance to several groups of antibiotics and can cause nosocomial infections (Maragakis et al., 2008; Peleg et al., 2008; Poirel et al., 2006; Tognim et al., 2004; Wroblewska et al., 2007). The spread of antimicrobial resistance among \text{A. baumannii} has raised an important challenge to our therapeutic approach. Unfortunately, there are no solid data regarding the susceptibility patterns of \text{A. baumannii} in Saudi Arabia or in the region of the Middle East (Al Johani et al., 2010; Al-Sweih et al., 2012; Memish et al., 2012; Mugnier et al., 2009; Park et al., 2010).

All 141 isolates identified as \text{A. baumannii} using 16S rRNA gene sequencing encoded \( \text{bla}_{\text{OXA-51}} \) gene which was not detected in any other Acinetobacter spp. tested. The data presented in this study support those of other studies demonstrating that \( \text{bla}_{\text{OXA-51}} \) is a cluster of constitutive oxacillinase genes found on the chromosome of \text{A. baumannii} (Turton et al., 2006b; Wroblewska et al., 2007). Therefore, \( \text{bla}_{\text{OXA-51}} \) may be used as a marker to identify \text{A. baumannii} especially in limited resource countries where more sophisticated technologies such as sequencing may not be available.

The data presented in this study reveal that the main ward for CRAB isolation is the ICU with the respiratory tract being the most common specimen source followed by wounds and urine. This is consistent with data presented by others where the major source of \text{A. baumannii} isolates was respiratory specimens followed by wounds (Ho et al., 2010; Kulah et al., 2010; Park et al., 2010).

The PFGE data revealed remarkable clonal diversity (44 clones among 46 strains). The low CDI indicates that each patient was infected by a different clone except in two clones. Although 56% of the isolates were recovered from the adult ICU, no single clone was related to that ward. The two groups of the indistinguishable strains were not clustered in time or place. Furthermore, environmental samples obtained as part of outbreak investigations identified no environmental source of these resistant CRAB isolates. Carbapenem resistance was not associated with any particular epidemiological molecular type. Based on the data presented, the interhospital transmission of CRAB isolates was apparently limited. These data also suggest that isolates were most probably brought into the hospital by patients, particularly as the King Fahad Specialist Hospital in Dammam is a referral hospital. Patients admitted to the hospital are referred from other ancillary hospitals and where most patients have usually been ex-
posed empirically to antibiotics for at least 1 week prior to admission in addition to over the counter antibiotics. The interhospital spread of CRAB isolates has been recognized as a major public health problem in several geographic areas. Therefore, active surveillance is needed to detect and prevent the dissemination of such isolates (Gur et al., 2008; Maragakis et al., 2008; Peleg et al., 2008; Poirel et al., 2006; Tognim et al., 2004).

Overall, the resistance rates were high for most antimicrobial agents with the exception of colistin (100% S) and to a lower extent to amikacin (73% S) and tigecycline (69.9% S). The resistance rates to gentamicin, fluoroquinolones, and β-lactam agents other than carbapenems including ceftazidime, piperacillin-tazobactam, and cefepime were more than 54%. The susceptibility pattern presented in this study is concordant with studies performed in the Kingdom of Saudi Arabia and other countries such as Kuwait, Qatar, Bahrain, United Arab Emirates, Iran, and Turkey (Al Johani et al., 2010; Al-Sweih et al., 2012; Andriamanantena et al., 2010; Feizabadi et al., 2008; Gur et al., 2008; Khan et al., 2012; Kulah et al., 2010; Memish et al., 2012; Mugnier et al., 2008; Mugnier et al., 2009).

Carbapenems have been the drug of choice in treating infections caused by A. baumannii. However, the number of carbapenem-resistant A. baumannii strains has increased recently (Maragakis et al., 2008; Peleg et al., 2008; Poirel et al., 2006; Tognim et al., 2004). The acquisition of carbapenem resistance in A. baumannii can be mainly due to the production of two types of carbapenem hydrolyzing enzymes OXA-β-lactamases (OBLs) and/or metallo-β-lactamases. The literature revealed that the OBLs are more prevalent to carbapenem resistance in A. baumannii (Maragakis et al., 2008; Peleg et al., 2008; Poirel et al., 2006; Tognim et al., 2004). The MBL Etest was performed and revealed that 43 isolates might harbor a metallo-β-lactamase. However, a PCR performed using specific primers for the genes encoding IMP and VIM was negative for all isolates. In agreement with our findings, Segal et al. found that 49 carbapenem-resistant A. baumannii clinical isolates were positive by MBL Etest screening and negative by PCR for IMP and VIM (Segal et al., 2005). In addition, several other studies published the same findings (Boo et al., 2009; Segal et al., 2005; Stoeva et al., 2008). The MBL Etest data can be explained by the data published on bla_{OXA-10} and bla_{OXA-14} (Danel et al., 2001). These enzymes, bla_{OXA-10} and bla_{OXA-14}, are found in two different forms: an active dimer and less active monomer. Divalent ions such as Zn^{2+}, Ca^{2+}, and Cu^{2+} are required for stabilization of the dimer. In the presence of the ion chelator EDTA, these enzymes are converted to a less active monomeric state associated with less carbapenem hydrolyzing activity (Danel et al., 2001). Therefore, the reduction of at least 3 double fold dilutions in IMP/IMPI observed using MBL Etest strips was not because of the MBL activity, and might be an indicator of the conversion of bla_{OXA-23} to the monomeric less active form. It is not known whether bla_{OXA-23} requires dimerization to be fully active and further workup is required to test this hypothesis. These data suggest that MBL Etest results should be cautiously interpreted when the organism tested is A. baumannii and a PCR test detecting genes encoding MBL and OXA is necessary.

Outbreaks caused by OBL-producing A. baumannii have been reported worldwide such as in Brazil, Spain, Southern Europe, Turkey, Korea, the Balkans, Argentina, and Iran (Gur et al., 2008; Khan et al., 2012; Nemec et al., 2008; Stoeva et al., 2008; Tognim et al., 2004; Wroblewska et al., 2007). Bla_{OXA-23} carbapenemase-producing A. baumannii are becoming globally widespread in Europe, South America, and Asia (Giannouli et al., 2010; Gur et al., 2008; Nemec et al., 2008; Wroblewska et al., 2007). The genetic analysis revealed that bla_{OXA-23} carbapenemase was detected in 37 (80.4%) of the CRAB strains associated with the ISAbal element. The ISAbal element was located upstream of the bla_{OXA-23} gene and was not associated with bla_{OXA-51} genes in bla_{OXA-23}-producing CRAB strains. This ISAbal element may act as a strong promoter and could be responsible for higher bla_{OXA-23} expression resulting in a high level of carbapenem resistance which correlates with the data published in the literature (Nemec et al., 2008; Segal et al., 2005; Turton et al., 2006a). In addition, these data suggest that the production of bla_{OXA-23} enzyme was the...
main mechanism of carbapenem resistance in bla_{OXA-23} producing CRAB isolates. However, the IS\textit{Aba} element was detected upstream of bla_{OXA-51} in only one strain of the non-bla_{OXA-23} producing CRAB isolates suggesting that bla_{OXA-51} was overexpressed downstream from the IS\textit{Aba} element and could be the reason for carbapenem resistance in this CRAB isolate. In support of these data, Turton et al. had shown that an IS\textit{Aba} element upstream of the chromosomally encoded OXA-51 β-lactamases can increase the expression of these genes, which are normally expressed at low level, and result in carbapenem resistance (Turton et al., 2006a). The mechanism of carbapenem resistance is not clear in the 8 CRAB strains encoding the bla_{OXA-51} gene as the sole carbapenemase gene which is not associated with the IS\textit{Aba} element. Further investigations are required to delineate the resistance mechanism in these isolates.

The OBLs are the main mechanism of carbapenem resistance in CRAB isolates investigated in this study which correlates with other studies conducted in Europe such as in Czech Republic and in the region such as in KSA, Bahrain, Qatar, UAE, and Turkey (Al-Sweih et al., 2012; Andriamananterena et al., 2010; Gur et al., 2008; Khan et al., 2012; Kulah et al., 2010; Memish et al., 2012; Mugnier et al., 2008; Mugnier et al., 2009; Nemec et al., 2008). However, these data are in contrast with a study conducted in Kuwait where the majority of carbapenem-resistant \textit{A. baumannii} produced bla_{IMP-1} and bla_{VIM-2} (Al-Sweih et al., 2012).

This study reveals that the resistance rates to carbapenems in \textit{A. baumannii} exceeds 32%, is in agreement with data published by other groups in KSA and neighboring countries such as Kuwait, UAE, Qatar, Turkey, and Bahrain (Al Johani et al., 2010; Al-Sweih et al., 2012; Andriamananterena et al., 2010; Gur et al., 2008; Khan et al., 2012; Kulah et al., 2010; Memish et al., 2012; Mugnier et al., 2008; Mugnier et al., 2009; Nemec et al., 2008). However, the data presented in this study are discordant with those of a study published in ARAMCO hospital, Dhrahn, KSA in 2007 where the resistance rate of \textit{A. baumannii} to imipenem was 3% (Al-Tawfiq et al., 2007). The discrepancy between the two studies can be due to the fact that ARAMCO hospital is a general hospital whereas our hospital is a tertiary hospital. In addition, the ARAMCO study was conducted on isolates collected from 1998 to 2004 compared to strains collected between 2010 and 2011 in our study. It is well documented in the literature that there has been a significant global increase in the incidence of carbapenem resistance in \textit{A. baumannii} over the years (Gur et al., 2008; Maragakis et al., 2008; Peleg et al., 2008; Poirel et al., 2006; Tognim et al., 2004).

In conclusion, this study reveals that multidrug resistant \textit{Acinetobacter baumannii} strains are spreading. Carbapenem resistance is significant in these isolates and bla_{OXA-23} carbapenemase is the most common gene responsible for such resistance.

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