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

Baha Abdalhamid[1], Hoda Hassan[1,2], Ahmad Itbaileh[1], Mahmoud Shorman[3]

[1]Department of Pathology and Laboratory Medicine, King Fahad Specialist Hospital, Dammam, Saudi Arabia;
[2]Department of Microbiology, Alexandria University, Alexandria, Egypt;
[3]Department of Internal Medicine, King Fahad Specialist Hospital, Dammam, Saudi Arabia,
Internal Medicine Department, Marshall University, Huntington, WV, USA

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 bla_{IMP} and bla_{VIM}. Among the 46 CRAB strains, 37 isolates harbored bla_{OXA-23} which was encoded downstream of IS*Aba1* and 1 isolate had IS*Aba1* encoded upstream bla_{OXA-51}. These data reveal that the interhospital transmission of CRAB isolates was apparently insignificant. Bla_{OXA-23} adjacent to IS*Aba1* 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.

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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;

Corresponding author
Baha Abdalhamid M.D., Ph. D., D (ABMM)
Department of Pathology and Laboratory Medicine
King Fahad Specialist Hospital
Dammam 31444
Kingdom of Saudi Arabia
E-mail: baha000@hotmail.com

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-lactamases with carbapenemase activity in *A. baumannii* are oxacillinases. OXA enzymes, encoded by bla_{OXA} 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 bla_{OXA-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 bla_{OXA-23}, bla_{OXA-27}, and bla_{OXA-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 IS*Aba1* element, belonging to the IS4 family, and has been detected upstream of bla_{OXA-23}, bla_{OXA-27}, and bla_{OXA-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 baumanni*, 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 *Apa*I-digested total chromosomal DNA prepared as described pre-

viously (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 $bla_{\text{OXA-51}}$ was performed to identify $bla_{\text{OXA-51}}$ in *A. baumannii* clinical isolates, ATCC BAA 747 strain, and in 6 previously identified clinical isolates of *A. lwoffii* and a clinical isolate of *A. junii*.

DNA extracts used as positive controls for bla_{IMP}, bla_{VIM}, $bla_{\text{-NDM1}}$, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-51}}$, and $bla_{\text{OXA-58}}$ 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 $bla_{\text{OXA-23}}$ 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*Aba1* element was identified using the IS*Aba1*F (CACGAATGCAGAAGTTG) and

TABLE 1 - *Primers used in this study*

Reference	*Amplicon Size*	*Sequence*	*Primer*
OXA24F	GGTTAGTTGGCCCCCTTAAA	246	Woodford *et al.*, 2006
OXA24R	AGTTGAGCGAAAAGGGGATT		
OXA23F	GATCGGATTGGAGAACCAGA	501	
OXA23R	ATTTCTGACCGCATTTCCAT		
OXA51F	TAATGCTTTGATCGGCCTTG	353	
OXA51R	TGGATTGCACTTCATCTTGG		
OXA58F	AAGTATTGGGGCTTGTGCTG	599	
OXA58R	CCCCTCTGCGCTCTACATAC		
ImpF	GAATAGAATGGTTAACTCTC	188	Mendes *et al.*, 2007
ImpR	CCAAACCACTAGGTTATC		
VIMF	GTTTGGTCGCATATCGCAAC	382	
VIMR	AATGCGCAGCACCAGGATAG		
OXA23FLF	GGAATTCCATGAATAAATATTTTACTTGC	822	Mugnier *et al.*, 2010
OXA23FLR	CGGGATCCCGTTAAATAATATTCAGGTC		
NDM1F	GAGATTGCCGAGCGACTTG	497	Chihara *et al.*, 2011
NDM1R	CGAATGTCTGGCAGCACACTT		

OXA23R primers and the IS*Aba1*F and OXA51R primers as described by Turton *et al.* (Turton *et al.,* 2006a). In addition, a PCR using IS*Aba1*F and IS*Aba1*R (CGACGAATACTATGACAC) primers (Turton *et al.,* 2006a) were conducted on a total of 5 representative carbapenem-susceptible *A. baumannii* strains to detect the IS*Aba1* 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 baumanni* 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).

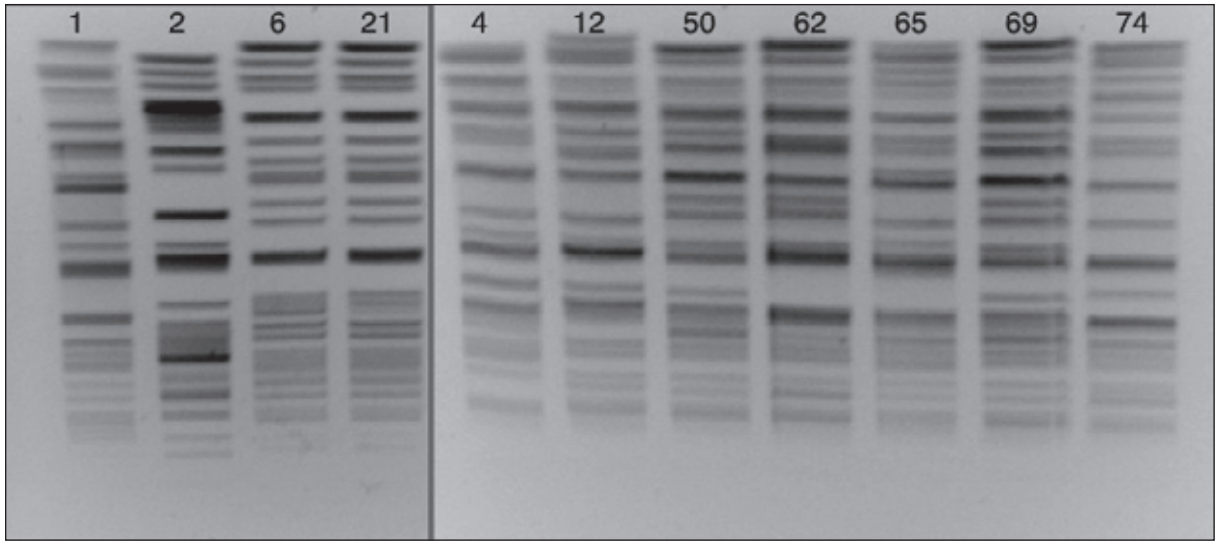

FIGURE 1 - *Pulse-field gel electrophoresis (PFGE) patterns in representative strains of CRAB. Strains were isolated from different hospital wards. Strains 6 and 21 and strains 50 and 69 are indistinguishable whereas the other strains are unrelated.*

PFGE revealed that the majority of the isolates had wild 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 $bla_{\text{OXA-51}}$ like. None of the *Acinetobacter* spp. other than *baumannii* (6 strains) harbored $bla_{\text{OXA-51-}}$ like.

In the 46 CRAB isolates, a PCR product of 501 bps correlating with $bla_{\text{OXA-23}}$ was detected in 80.4% (n=37) of the isolates and was confirmed to be $bla_{\text{OXA-23}}$ by sequencing the full length PCR product (822 bps). All 37 CRAB isolates that were PCR positive for $bla_{\text{OXA-23}}$ had an expected band of 1.6 kb in a PCR using IS*Aba1*F and OXA23R. Although all of these isolates were PCR positive for $bla_{\text{OXA-51}}$, none gave a band in the PCR using IS*Aba1*F and OXA51R.

The IS*Aba1* element was detected upstream $bla_{\text{OXA-51}}$ in only 1 $bla_{\text{OXA-23}}$ non-producing CRAB isolates with an expected band of 1.2 kb. There were 8 isolates encoding only $bla_{\text{OXA-51}}$ gene and was not associated with the IS*Aba1* element. The IS*Aba1* element was not detected in any of the 5 representative carbapenem susceptible *A. baumannii* strains. *Bla*-$_{\text{NDM1}}$, *bla*-$_{\text{IMP}}$ or *bla*-$_{\text{VIM}}$ metallo-β-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 *A. baumannii* has raised an important challenge to our therapeutic approach. Unfortunately, there are no solid data regarding the susceptibility patterns of *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 *A. baumannii* using 16S rRNA gene sequencing encoded $bla_{\text{OXA-51}}$ gene which was not detected in any other *Acinetobacter* spp. tested. The data presented in this study support those of other studies demonstrating that $bla_{\text{OXA-51}}$ is a cluster of constitutive oxacillinase genes found on the chromosome of *A. baumannii* (Turton *et al.*, 2006b; Wroblewska *et al.,* 2007). Therefore, $bla_{\text{OXA-51}}$ may be used as a marker to identify *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 *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 $bla_{\text{OXA-10}}$ and $bla_{\text{OXA-14}}$ (Danel *et al.,* 2001). These enzymes, $bla_{\text{OXA-10}}$ and $bla_{\text{OXA-14}}$, 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 $bla_{\text{OXA-23}}$ to the monomeric less active form. It is not known whether $bla_{\text{OXA-23}}$ 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). $Bla_{\text{OXA-23}}$ 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 $bla_{\text{OXA-23}}$ carbapenemase was detected in 37 (80.4%) of the CRAB strains associated with the IS*Aba1* element. The IS*Aba1* element was located upstream of the $bla_{\text{OXA-23}}$ gene and was not associated with $bla_{\text{OXA-51}}$ genes in $bla_{\text{OXA-23}}$-producing CRAB strains. This IS*Aba1* element may act as a strong promoter and could be responsible for higher $bla_{\text{OXA-23}}$ 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 $bla_{\text{OXA-23}}$ enzyme was the

main mechanism of carbapenem resistance in $bla_{\text{OXA-23}}$ producing CRAB isolates. However, the IS*Aba1* element was detected upstream of $bla_{\text{OXA-51}}$ in only one strain of the non-$bla_{\text{OXA-23-}}$ producing CRAB isolates suggesting that $bla_{\text{OXA-51}}$ was overexpressed downstream from the IS*Aba1* 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*Aba1* 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 $bla_{\text{OXA-51}}$ gene as the sole carbapenemase gene which is not associated with the IS*Aba1* 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; Andriamanantena *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 *A. baumannii* produced $bla_{\text{IMP-1}}$ and $bla_{\text{VIM-2}}$ (Al-Sweih *et al.,* 2012).

This study reveals that the resistance rates to carbapenems in *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; Andriamanantena *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 *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 *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 *Acinetobacter baumannii* strains are spreading. Carbapenem resistance is significant in these isolates and $bla_{\text{OXA-23}}$ carbapenemase is the most common gene responsible for such resistance.

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