African Journal of Biotechnology Vol. 10(64), pp. 14186-14196, 19 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2291
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Genetic basis of carbapenem resistance in *Acinetobacter* clinical isolates in Saudi Arabia

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Accepted 12 October, 2011

Carbapenem-hydrolyzing oxacillinases are reported increasingly in *Acinetobacter baumannii*. Here we report the contribution of carbapenem-hydrolyzing oxacillinases genes to carbapenem resistance in clinical *Acinetobacter baumannii* strains in Saudi Arabia. Forty non-repetitive clinical *A. baumannii* strains were isolated and identified from 40 patients, hospitalized in various wards in King Khalid University and Armed Forces Hospitals (Riyadh, Saudi Arabia). Antibiotic susceptibility testing indicated that most isolates (65 to 100% of the total strains) were resistant to β-lactams antibiotics with minimum inhibitory concentrations (MICs) ranged from low to very high values. In addition, 65 and 67.5% of the total isolated clinical strains were resistant to carbapenem antibiotics, including imipenem and meropenem, respectively. Based on antibiotic susceptibility, it was possible to divide the isolated clinical *A. baumannii* strains into four phenotypes clusters, I, II, III and IV, with multiple antibiotic resistance of > 90% (with very high MICs), 80 to 89% (with high MICs), 70 to 79% (with moderate MICs), and 40 to 69 (with moderate to low MICs), of the total antibiotics (n = 17), respectively. The results of polymerase chain reaction (PCR) products analysis reveals that the major groups of oxacillinases genes including *bla*$_{OXA-23}$, *bla*$_{OXA-24}$, and *bla*$_{OXA-58}$ were detected in 72.5% (n = 29), 45% (n = 18) and 37.5% (n = 15) of the isolated *A. baumannii* strains, respectively. In addition, analysis of the prevalence of different oxacillinases genes in different antibiotics-based phenotypes clusters, revealed that cluster I harbored the highest distribution of resistant genes, which could explain the extremely multiple antibiotic resistance phenotype within the strains of this cluster.

Key words: *Acinetobacter baumannii*, oxacillinases, multi drug resistance, β-lactam.

INTRODUCTION

Acinetobacter baumannii has emerged as a major nosocomial opportunistic pathogen in hospital infections worldwide and is ranked second after *Pseudomonas aeruginosa* among nosocomial pathogens of aerobic nonfermentative gram-negative bacilli (Qi et al., 2008; Bonnin et al., 2011). *A. baumannii* causes urinary and respiratory tract infections, endocarditis, meningitis, burn infections, and wound sepsis, especially in intensive care units (ICUs) and in immunocompromised patients and is associated with high morbidity and mortality rate (Chen et al., 2010). In addition to ubiquity in nature and its ability to survive for long periods in adverse environmental conditions, *A. baumannii* is characterized by its potential to acquire antimicrobial resistance genes rapidly, leading to multidrug resistance (Park et al., 2010). Furthermore,

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Abbreviations: MICS, Minimum inhibitory concentrations; **PCR**, polymerase chain reaction; **ICUs,** Intensive Care Units; **MDR,** multidrug resistant; **CHDLs,** carbapenem-hydrolyzing oxacillinases; **CLSI,** Clinical and Laboratory Standard Institute.

the genetic flexibility and high adaptability of this organism have resulted in the rapid and global emergence over the last few years of multidrug resistant (MDR) *A. baumannii* strains resistant to most classes of antimicrobial drugs, including broad-spectrum β-lactams, carbapenems, aminoglycosides, and fluoroquinolones (Bogaerts et al., 2010; Zarrilli et al., 2008). Recently, strains displaying resistance to all commercially available antimicrobial drugs have been reported, making treatment of these infections difficult and in some cases impossible (Park et al., 2010; Tian et al., 2011). *A. baumannii* may develop resistance to carbapenems (for example, imipenem and meropenem), which have become the drugs of choice against *Acinetobacter* infections in many healthcare centers. Various mechanisms are involved in resistance to carbapenems including decreased membrane permeability because of porin modifications or reduced expression, over expression of efflux pumps, and production of carbapenemases, such as metallo-β-lactamase or carbapenem-hydrolyzing oxacillinases (CHDLs), (Perez et al., 2007; Peleg et al., 2008).

However, carbapenem resistance has been correlated mainly with the acquisition of Class D β-lactamases CHDLs. Three main acquired CHDL gene clusters have been identified in *A. baumannii*, represented by the *blaOXA-23*-, *blaOXA-24*- and *blaOXA-58*-like genes (Zarrilli et al., 2008). The emergence of carbapenem resistance in *A. baumannii* has been reported worldwide including hospitals in Europe, North America, Argentina, Brazil, China, Taiwan, Hong Kong, Japan and Korea and from areas as remote as Tahiti in the South Pacific (Nishio et al., 2004; Naas et al., 2005; Lee et al., 2006; Liu et al., 2006; Poirel et al., 2007; Qi et al., 2008; Kim et al., 2010). Unfortunately, the emergence of MDR nosocomial *A. baumannii* has also reported in several hospitals in Saudi Arabia, including King Faisal Specialist Hospital (Riyadh), King Abdulaziz University Hospital (Jeddah), Dhahran Health Center (Dhahran) and King Khalid University Hospital (Riyadh), (Eltahawy and Khalaf, 2001; Hanan et al., 2003; Bukhary et al., 2005; Al-Tawfiq et al., 2007). It has been established that there is difference in the antibiotic resistance rates of *A. baumanii* in different areas in the world, which is mostly due to factors such as antimicrobial use patterns, infection control practices and climate (Perez et al., 2007; Peleg et al., 2008). Therefore, extensive research on the antibiotic susceptibility profile and mechanism of resistance of local *A. baumanii* is strongly needed to monitor the emergence of resistance and its mechanism to commonly used antibiotics. Thus, the main objective of the present study was to investigate the distribution of carbapenem-hydrolyzing oxacillinases gene in local clinical MDR *A. baumanii* strains. This work represents one of the first studies at the molecular level of nosocomial MDR *A. baumanii* in Saudi Arabia.

MATERIALS AND METHODS

Clinical samples collection and bacterial strains identification

Different clinical specimens were collected from patients hospitalized in the intensive care unit, surgery, medicine, neurology and urology wards in King Khalid University and Armed Forces Hospitals (Riyadh, Saudi Arabia), during the period between January 2007 and 2009. The samples included skin ulcers swabs, respiratory specimens, urine, blood, pus samples and catheter tips. The specimens were collected under sterile conditions using sterile cotton swabs, syringes and transport media, and were transferred to the laboratory in cold box within 1 to 2 h. The isolated clinical bacterial strains were identified using conventional biochemical tests, API 20NE (Biomérieux) Vitek 2 and/or MicroScan Walkaway® automated systems (Dade Behring, CA), as previously reported (Dalla-Costa et al., 2003; Heritier et al., 2005; Jeon et al., 2005; Villalon et al., 2011).

Antimicrobial agent and determination of MICs

Susceptibility of the isolated non- repetitive clinical *A. baumannii* strains toward various antimicrobial agents (n = 17) was investigated using agar disc diffusion method (Poirel et al., 2007; Bonnin et al., 2011). All bacterial strains were sub-cultured in fresh Mueller-Hinton agar plates for 24 h at 37°C. After the incubation period, the cells were collected using sterile loop and suspended in sterile saline solution (0.9% NaCl) to be equivalent to 0.5 McFarland standards. The cells suspensions were inoculated into Mueller-Hinton agar plates (Difco), using sterile cotton swabs, and various antibiotic discs were placed (in duplicate) carefully on the agar plates surfaces and incubated for 24 to 48 h at 37°C. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 10390 were used as control microorganisms. Inhibition zone diameter indicating susceptibility and resistance were evaluated according to clinical and laboratory standard institute (CLSI) guideline (Poirel et al., 2007; Peleg et al., 2008). The MICs of various antibiotic (n = 17) against the isolated *A. baumannii* strains (n = 40) were determined using MicroScan Walk-Away® automated system according to the manufacturer's instructions, and were confirmed using E-test (Bertini et al., 2007; Poirel et al., 2007). E-test strip of various antibiotic were placed (in triplicate) carefully on the inoculated agar plates surfaces and incubated for 24 to 48 h at 37°C. MIC values were determined as the lowest concentration of antibiotic able to inhibit the bacterial growth and the results were interpreted as recommended by CLSI (Peleg et al., 2008).

Identification of the oxacillinases genes

PCR was used for detection of the major groups of oxacillinases genes including bla_{OXA-23}-, bla_{OXA-24}-, and bla_{OXA-58}-like genes, using modification of previously reported methods (Bertini et al., 2007; Poirel et al., 2008; Qi et al., 2008). The list of the primers specific for different genes used in this study is presented in Table 1. *A. baumannii* strains (n = 40) were grown overnight in 5 ml broth medium at 37ºC and the cell biomass were collected by centrifugation at 7000 × *g* for 10 min, and washed twice using sterile distilled water. Total DNA was extracted using DNeasy blood and tissue kits (Qiagen) following the manufacturer's instructions. Gradient PCR was initially carried out to determine the optimum annealing temperature of each primer, using a gradient of annealing temperatures ranged from 48 to 64ºC. Then, conventional PCR was performed, using optimum annealing temperatures, for detection of different oxacillinases genes in the isolated *A.*

Table 1. List of primers used in this study.

Gene	Sequence (5'-3')	Reference
bla_{OXA-23}-F	GATGTGTCATAGTATTCGTCGT	Jeon et al., 2005
bla_{OXA-23}-R	TCACAACAACTAAAAGCACTG T	Jeon et al., 2005
bla_{OXA-24}-F	ATGAAAAAATTTATACTTCCTATATTCAGC	Jeon et al., 2005
bla_{OXA-24}-R	TTAAATGATTCCAAGATTTTCTAGC	Jeon et al., 2005
bla_{OXA-58}-F	AAGTATTGGGGCTTGTGCTG	Qi et al., 2008
bla_{OXA-58}-R	CCCCTCTGCGCTCTACATAC	Qi et al., 2008

baumannii strains (n = 40). PCR amplification was performed in a final reaction volume of 50 µl and the reaction mixture contained 25 µl of GoTaq® Green Master Mix (2X), (Promega, cat no. 7122), 1 µl of upstream primer (10 µM), 1 µl of downstream primer (10 µM), 5 µl deoxyribonucleic acid (DNA) template (200 ng) and 18 µl of nuclease-free water. The PCR reaction was run for 35 cycles in a DNA thermal cycler under the following thermal profile: Initial denaturation at 95°C for 5 min, denaturation at 95°C for 1 min, primers annealing at 52°C for 1 min and extension at 72°C for 1.5 min. The final cycle included extension for 10 min at 72°C to ensure full extension of the products. PCR products were run in agarose gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen), and sequenced using an automated sequencer (Research center, King Faisal Hospital, Riyadh, Saudi Arabia).

RESULTS AND DISCUSSION

Isolation and identification of *A. baumannii*

Enrichment and isolation of *A. baumannii* from the collected clinical specimens resulted in isolation of 40 non-repetitive clinical bacterial strains. The isolates grown on blood agar medium for 24 to 48 h at 37°C showed non-haemolytic colonies that were about 2 to 3 mm in diameter. The colonies were not pigmented when they grew on blood agar; however, they produced a pale yellow to white-greyish pigment on Muller Hinton agar medium. Culture grown in liquid medium for 24 h showed gram negative, non-motile, non-spore forming coccobacilli. The cell and colony morphology of the isolated bacterial strains were inconsistent with those reported for the typical strain of *A. baumannii* (Constantiniu et al., 2004; Perez et al., 2007; Peleg et al., 2008). Furthermore, all isolated strains were able to grow at 44°C, which is a characteristic feature of *A. baumannii* (Constantiniu et al., 2004; Peleg et al., 2008). The isolated clinical bacterial strains (n = 40) were identified using different biochemical tests, automated MicroScan Walk-Away® and/or Vitek 2 systems, and API 20NE, which identified the isolated bacterial strains as *A. baumannii* (n = 40). The results indicated that all bacterial isolates (n = 40) were non-motile, non-hemolytic, oxidase-negative, catalase positive and gelatinase negative. In addition, they were not able to hydrolyze esculin, and most strains were not able to reduce nitrate (97.5% of the total isolates), indole production (97.5%), glucose fermentation (95%), arginin dihydrolase (92.5%), and urease production (95%). However, there was significant variation among the isolated strains regarding assimilation of different carbon sources. All of these biochemical characteristics of the isolated bacterial strains are in consistent with those reported for *A. baumannii* (Constantiniu et al., 2004; Kulah et al., 2010). The identified *A. baumannii* strains were designated as *A. baumannii* strain KSU-DM1 to *A. baumannii* strain KSU-DM40.

Origin and distribution of isolated clinical *A. baumanii* strains

Forty non-repetitive clinical *A. baumanii* strains were isolated from 40 patients, including 23 males and 17 females. As shown in Figure 1, it was found that the highest recovery of *A. baumannii* strains was from wounds infections (25%, n = 10) and urine samples (25%, n = 10), followed by blood (12.5%, n = 5), catheter tips (10%, n = 4), bronchial fluid (7.5%, n = 3) and tracheal aspirates specimens (5%, n = 2), respectively. These results are in consistent with that reported by Kulah et al. (2010) of high recovery of *A. baumannii* from wounds infections. However, in his study, the highest *A. baumannii* recovery was from tracheal aspirates (32%), followed by wound swabs (22%), blood (14%), bronchoalveolar specimens (11%) and urine, sterile fluids, catheter tips, abscess and sputum (each < 5%), respectively (Kulah et al., 2010). In another study on 45 patients (27 men and 18 women), *A. baumannii* were recovered from respiratory tract (n = 24), wound (n = 15), urine (n = 4) and catheter tips (n = 2), (Ho et al., 2010). Park et al. (2010) reported isolation of 30 MDR *A. baumannii*. Sites from which they were recovered included the respiratory system (20; 77%), soft tissue (3; 12%) and intra-abdominal (2; 8%); one case had primary bacteraemia (Park et al., 2010). Furthermore, analysis of the relationship between incidence of *A. baumannii* and patients age, indicated that there was increase of the pathogen incidence with patients' age, with highest incidence in patients of 61 to 70-years old and mean age of 61.6 years (Figure 2). This finding is not surprising, as MDR *A. baumannii* infections tend to occur in old

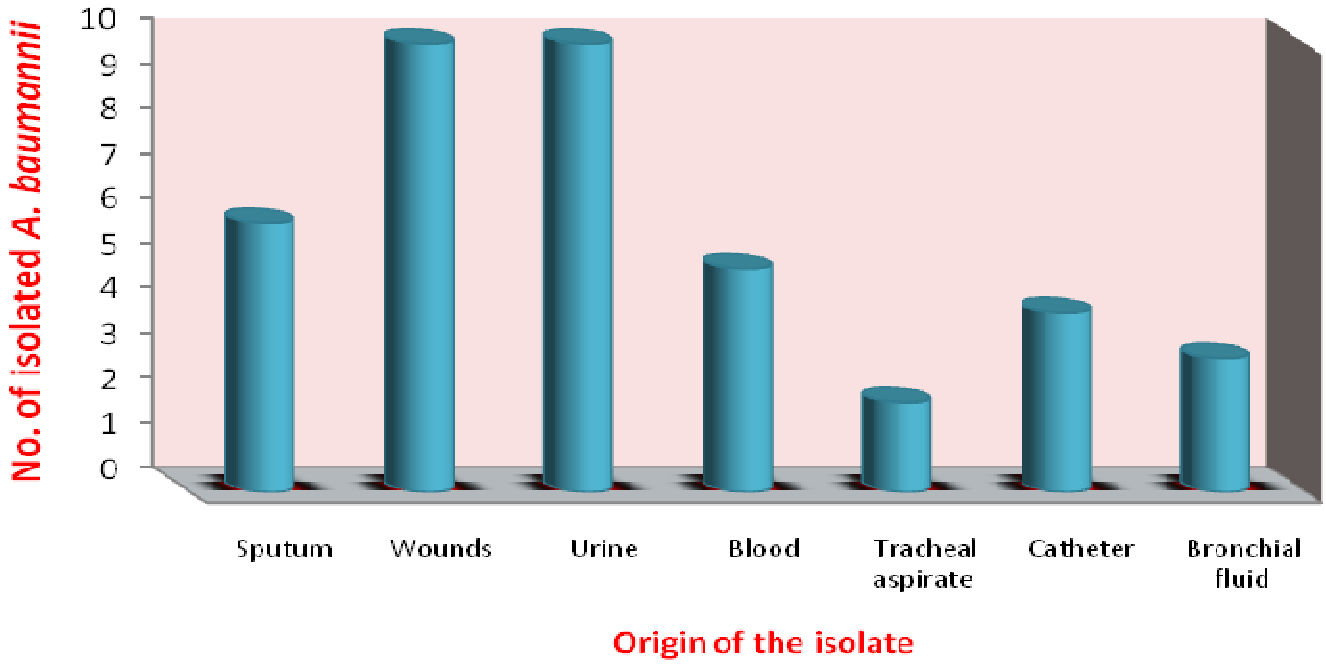

Figure 1. Origin of the isolated clinical *A. baumannii* strains. Different specimens were collected from patients hospitalized in the intensive care unit, surgery, medicine, neurology, and urology wards in King Khalid University and Armed Forces Hospitals (Riyadh, Saudi Arabia), over the period between January 2007 and January 2009.

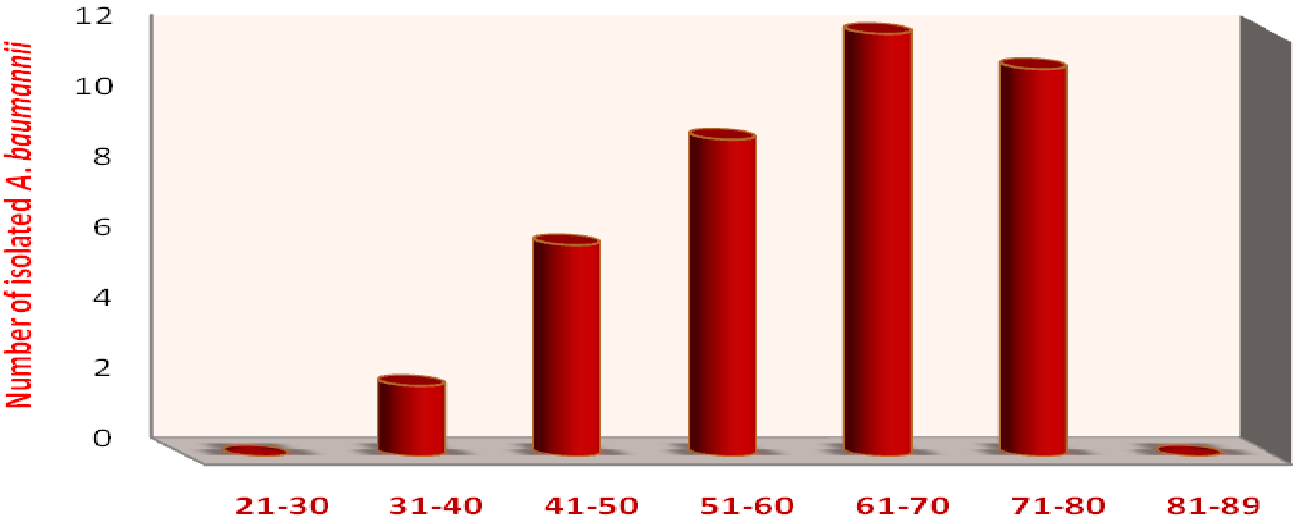

Figure 2. Relationship between incidence of *A. baumannii* and patients age.

immune-suppressed patients, in patients with serious underlying diseases and in those subjected to invasive procedures and treated with broad-spectrum antibiotics (Perez et al., 2007; Peleg et al., 2008; Park et al., 2010). In a previous study on 26 patients with drug resistant *A. baumannii*, 20 were men and 6 were women, the mean age was 63 ± 14 years (Park et al., 2010). In another study on 45 patients with MDR *A. baumannii* reported by Ho et al. (2010), the mean age of patients was 73.7 years (range: 29 to 101 years).

Antibiotic susceptibility

Investigation of susceptibility of the isolated nosocomial *A. baumannii* strains (n = 40) toward various β-lactams

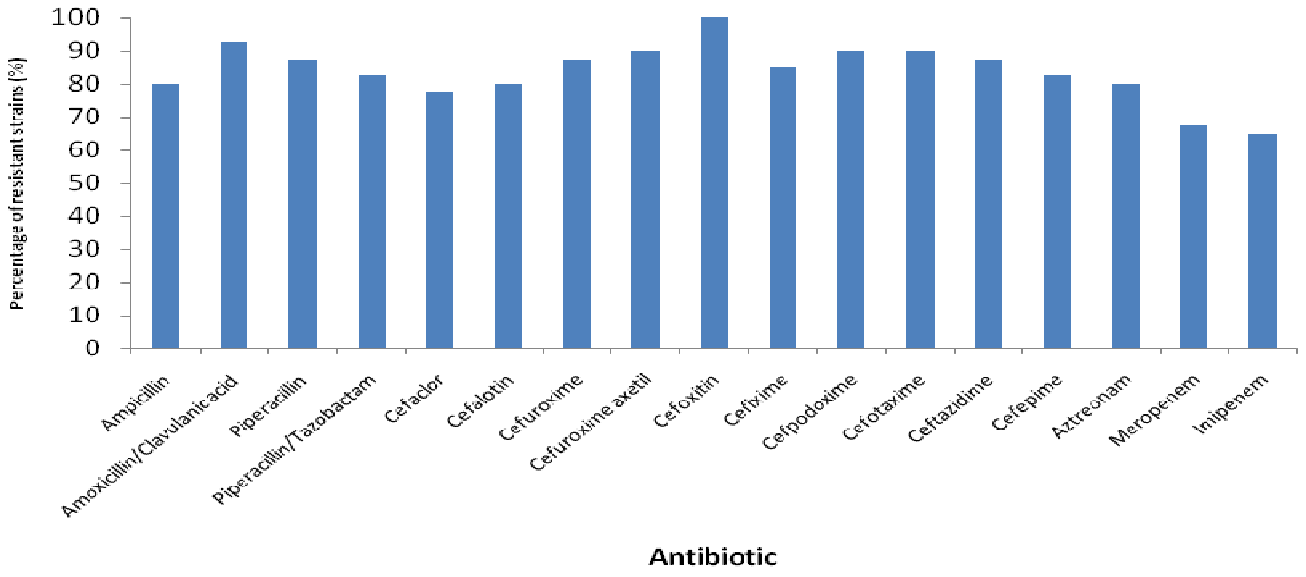

Figure 3. Summary of susceptibility of isolated clinical *A. baumannii* strains (n=40) toward various antibiotics (n=17).

antibiotics (n = 17), and determination of the MICs, revealed that there was high level of antibiotics resistance among the isolated *A. baumannii* strains (Figure 3 & 4). The results indicated that most isolates (65 to 100% of the total strains) were highly resistant to β-lactams antibiotics with MICs ranged from low to very high values. Relatively similar results have been reported by Lin et al. (2010), who indicated that 83% (44/53) of *A. baumannii* isolated strains from Taiwanese hospitals were resistant to β-lactams antibiotics. In addition, Bogaerts et al. (2010) reported emergence of MDR *A. baumannii* with high resistance to β-lactams antibiotics of up to 100% of the strains isolated from patients in Belgium. Others have also reported similar results of high resistance of *A. baumannii* to β-lactams antibiotics (Marque et al., 2005; Koh et al., 2007; Poirel et al., 2007, Tian et al., 2011). In addition, the isolated clinical *A. baumannii* strains demonstrated relatively high level of resistance to carbapenem antibiotics, including imipenem and meropenem, with resistant strains of 65 and 67.5% of the total isolated strains, respectively. Carbapenem antibiotics have become the drugs of choice against *A. baumannii* infections in many healthcare centers, but are slowly being compromised by the emergence of carbapenem-hydrolyzing β-lactamases of molecular classes B and D (Dalla-Costa et al., 2003; Poirel et al., 2010).

In a previous study, *A. baumannii* strains isolated from 12 different cities in Europe, of which 90% (n = 43) and 65% (n = 31) were resistant to imipenem and meropenem, respectively (Marque et al., 2005). Koh et al. (2007) reported isolation of *A. baumannii* isolates, 21.2% of which were resistant to imipenem. However, the results of antimicrobial susceptibility testing of 193 non-repetitive *A. baumannii* strains isolated in Korea, showed less detection (26.9%, 52 of 193) of imipenem-resistant isolates (Jeon et al., 2005). In our work, out of 17 antibiotics tested, cefoxitin was the most non-effective *in vitro* against any strain, as 100% of the isolated *A. baumannii* strains were resistant to this antibiotic. Furthermore, the isolated clinical *A. baumannii* strains showed multiple antibiotics resistance ranged from 7 to 17 different antibiotics out of 17 tested antibiotics. *A. baumannii* strains KSU-DM7, KSU-DM15, KSU-DM17, KSU-DM21, KSU-DM26, KSU-DM29, KSU-DM30, KSU-DM36, KSU-DM38 and KSU-DM39 were the most resistant strains, showing resistance to 100% of tested antibiotics (n = 17). In addition, the most sensitive strains were *A. baumannii* KSU-DM24 and KSU-DM33, which were sensitive to 58.8% (n = 11) of the used antibiotics (n = 17). Based on antibiotic susceptibility and in combination with the corresponding MIC values of each antibiotic, it was possible to divide the isolated clinical *A. baumannii* strains (n = 40) into four phenotypes. They were designated as phenotype cluster I (n = 16), cluster II (n = 11), cluster III (n = 9), and cluster IV (n = 4), with multiple antibiotic resistance of > 90% (with very high MICs), 80 to 89% (with high MICs), 70 to 79% (with moderate MICs), and 40 to 69% (with moderate to low MICs), of the total antibiotics (n = 17), respectively (Table 2).

Genetic basis of carbapenem resistance in the clinical *A. baumannii* strains

Carbapenem resistance has been correlated mainly with the acquisition of class D β-lactamases (CHDLs),

Table 2. Antibiotic susceptibility-based phenotypes of the isolated clinical *A. baumannii* strains (n=40).

Acinetobacter *baumannii* strain	Phenotype	Antibiotic resistance (%)	No of strains	MICs average
KSU-DM4, KSU-DM23, KSU-DM25, KSU-DM27, KSU-DM35, KSU-DM40, KSU-DM7, KSU-DM15, KSU-DM17, KSU-DM21, KSU-DM26, KSU-DM29, KSU-DM30, KSU-DM36, KSU-DM38, KSU-DM39	Cluster I	> 90	16	Very high
KSU-DM39, KSU-DM14, KSU-DM20, KSU-DM5, KSU-DM10, KSU-DM11, KSU-DM13, KSU DM18, KSU-DM19, KSU-DM32, KSU-DM34, KSU-DM37	Cluster II	80-89	11	High
KSU-DM1, KSU-DM2, KSU-DM6, KSU-DM8, KSU-DM28, KSU-DM3, KSU-DM9, KSU-DM12, KSU-DM22	Cluster III	70-79	9	Moderate
KSU-DM24, KSU-DM33, KSU-DM16, KSU-DM31	Cluster IV	40-69	4	Low

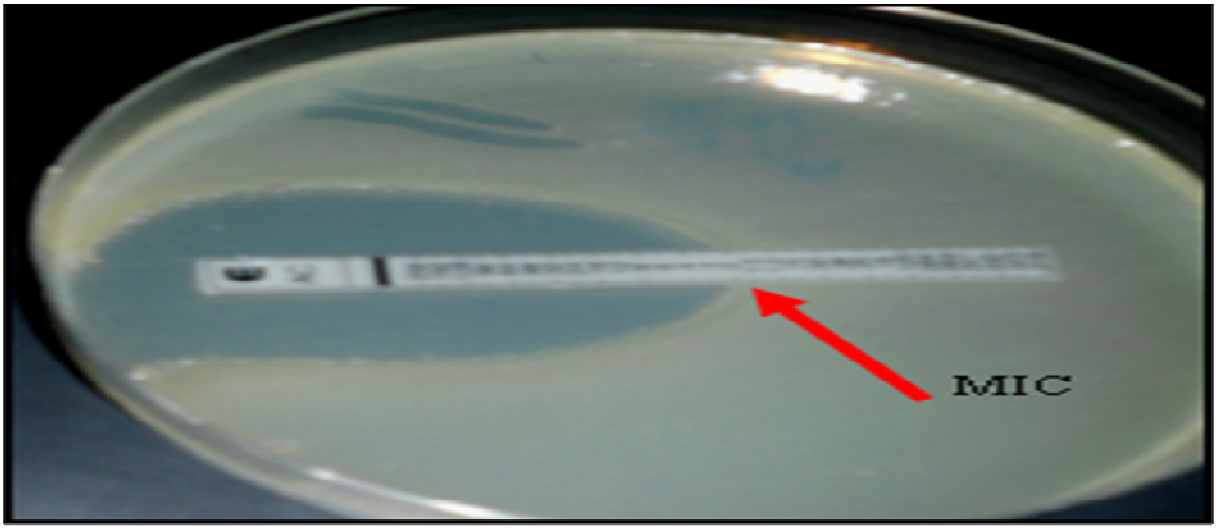

Figure 4. Determination of MICs values of various antibiotics using E-test. MICs values were determined as the lowest concentration of antibiotic able to inhibit the bacterial growth (red arrow).

(oxacillinases). Three main acquired CHDL gene clusters have been identified in *A. baumannii*, represented by the *blaOXA-23*-, *blaOXA-24*- and *blaOXA-58*-like oxacillinases genes (Zarrilli et al., 2008). PCR was used for detection of different oxacillinases genes responsible for carbapenem resistance in the isolated clinical *A. baumannii* and series of primers were selected based on the conservative region in the major groups of oxacillinase genes including bla_{OXA-23}, bla_{OXA-24}, and bla_{OXA-58}. Gradient PCR was initially used to determine the annealing temperature for each primer and then conventional PCR was used. The results of PCR products analysis revealed the detection of bla_{OXA-23} with the correct amplicons size of about 1058 bp and sequence identity (Jeon et al., 2005) in 72.5% (n = 29) of the total isolated *A. baumannii* strains (n = 40) (Figure 5). This result is relatively similar to that obtained by other scientists, who reported detection of bla_{OXA-23} in 69.2% of the isolated *A. baumannii* strains (36/52) isolated from hospitals in Republic of Korea (Jeon et al., 2005), and in 77% of *A. baumannii* strains isolated from hospitals in South Korea (Park et al., 2010). However, much higher prevalence of bla_{OXA-23} (87.5%; 28/32) was reported in *A. baumannii* strains isolated from patients hospitalized in Sydney Hospital, Australia (Mak et al., 2009), in 91% of *A. baumannii* strains isolated from Singapore General Hospital (Koh et al., 2007), and 100% (n = 24) of strains isolated from a healthcare region in Hong Kong, (Ho et al., 2010).

The results also revealed that bla_{OXA-24} was detected with the correct amplicon size of 825 bp and sequence identity (Jeon et al., 2005), in 45% (n = 18) of the total isolated clinical *A. baumannii* strains (n = 40), (Figure 6). The prevalence of bla_{OXA-24} in the clinical *A. baumannii*

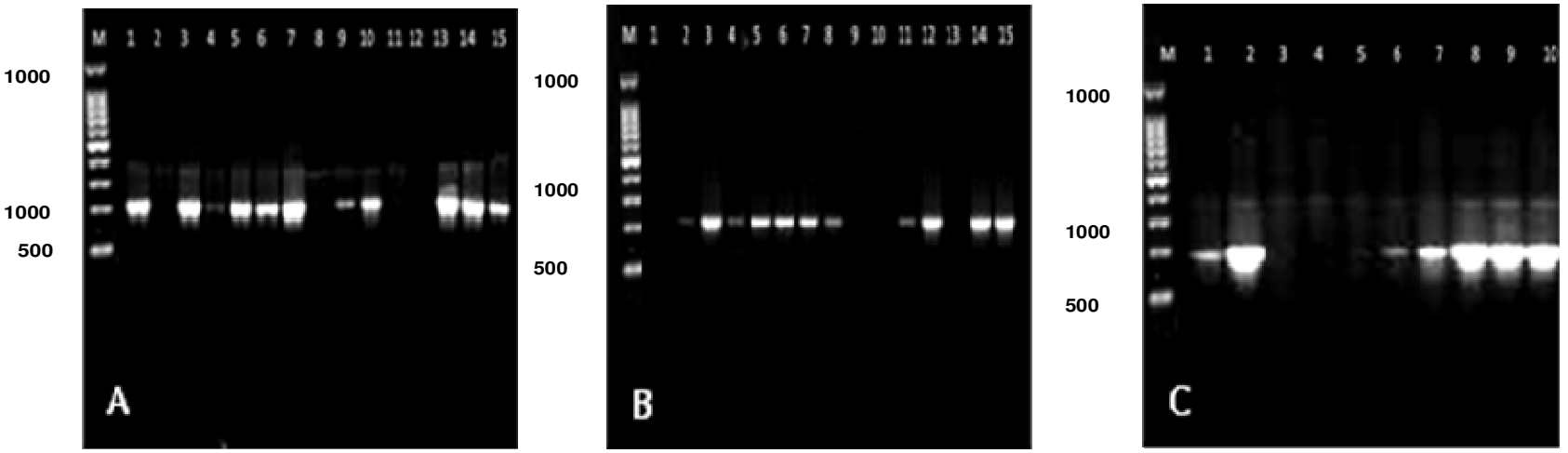

Figure 5. Agarose gel electrophoresis of the amplified *bla*$_{OXA-23}$ gene. 15 µl of the PCR product was separated in 1% agarose containing ethidium bromide solution (1 µg/ml), and visualized using Gel Documentation System; (**A**) M: 1 kbp DNA ladder. Lanes 1 to 15: *A. baumannii* strain KSU-DM1 to KSU-DM15; (**B**) M: 1 kbp DNA ladder**.** Lanes 1 to 15**:** *A. baumannii* strain KSU-DM16 to KSU-DM30; (**C**) M: 1 kbp DNA ladder**.** Lanes 1 to 10: *A. baumannii* strain KSU-DM31 to KSU-DM40.

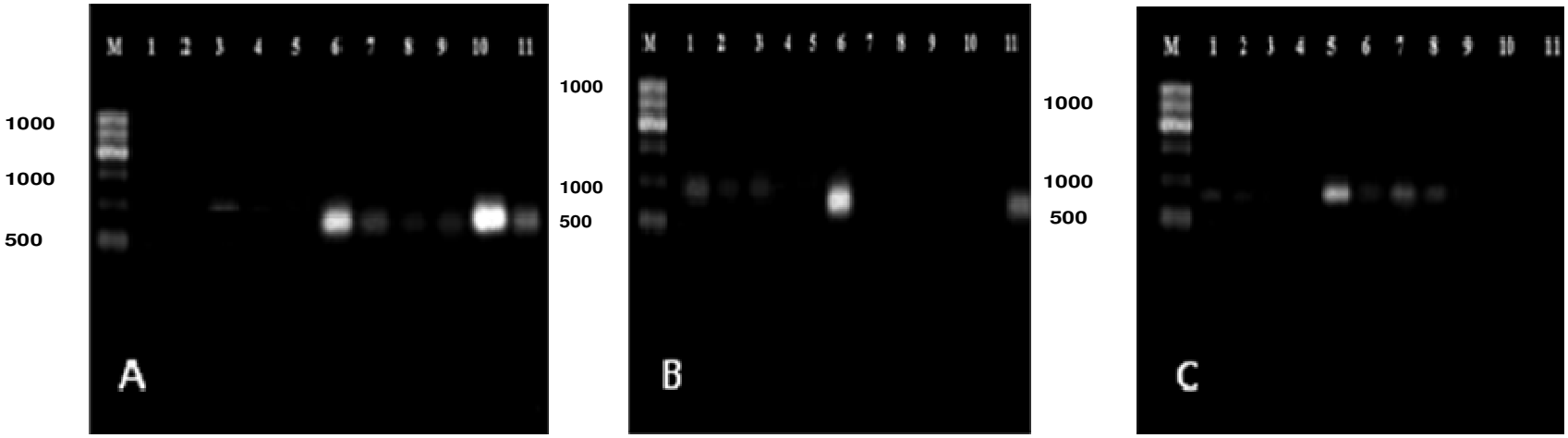

Figure 6. Agarose gel electrophoresis of the amplified *bla*$_{OXA-24}$ gene. 15 µl of the PCR product was separated in 1% agarose containing ethidium bromide solution (1 µg/ml), and visualized using gel documentation system; (**A**) M: 1 kbp DNA ladder. Lanes 1 to 11: *A. baumannii* KSU-DM1 to KSU-DM11 strains; (**B**) M: 1 kbp DNA ladder**.** Lanes 1 to 11: *A. baumannii* strain KSU-DM12 to KSU- DM22; (**C**) M: 1 kbp DNA ladder. Lanes 1 to 11: *A. baumannii* strain KSU-DM23 to KSU-DM33.

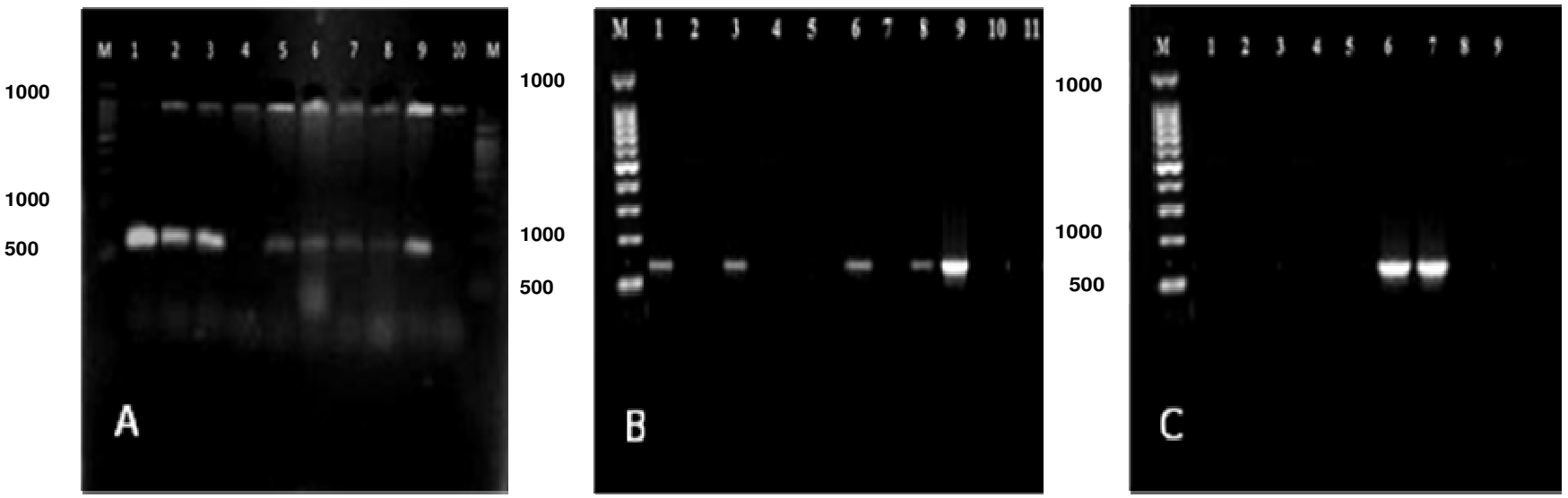

Figure 7. Agarose gel electrophoresis of the amplified *bla*$_{OXA-58}$ gene. 15 µl of the PCR product was separated in 1% agarose containing ethidium bromide solution (1 µg/ml), and visualized using gel documentation system; (**A**) M: 1 kbp DNA ladder**.** Lanes 1 to 10: *A. baumannii* strain KSU-DM1 to KSU-DM10; (**B**) M: 1 kbp DNA ladder. Lanes 1 to 11: *A. baumannii* strain KSU-DM21 to KSU- DM31; (**C**) M: 1 kbp DNA ladder. Lanes 1 to 9: *A. baumannii* strain KSU-DM32 to KSU-DM40.

strains is much higher than previously reported in clinical *A. baumannii* (Kulah et al., 2010; Park et. al., 2010). *bla*$_{OXA-58}$ gene has been also amplified with the correct amplicon size of 599 bp and sequence identity (Qi et al., 2008), in 37.5% (n = 15) of the total isolated clinical *A. baumannii* strains (Figure 7). Marque´ et al. (2005) has reported detection of *bla*$_{OXA-58}$ in 47.8% (22/46) of clinical *A. baumannii* strains isolated from different cities in Europe. Much higher prevalence of *bla*$_{OXA-58}$ of 79% (23/29) and 100% (19/19) of clinical *A. baumannii* strains has been previously reported (Poirel et al., 2008; Kulah et al., 2010). However, *bla*$_{OXA-58}$ was not detected in any of the resistant *A. baumannii* strains (Park et al., 2010). Table 3 shows the distribution of various antibiotic resistance carbapenemases genes in the isolated clinical *A. baumannii* strains (n = 40). Analysis of the prevalence of different oxacillinases genes in different antibiotics-based phenotypes clusters (Table 4) revealed that cluster I harbored the highest distribution of resistant genes, which could explain the extremely multiple antibiotic resistance phenotype of this cluster. *bla*$_{OXA-23}$ was detected in 87.5, 81.8, 55.6 and 25% in the strains of cluster I, II, III and IV, respectively. In addition, it was found that *A. baumannii* strains, showing the highest multiple antibiotics resistance (17/17), harbor the three carbapenem-hydrolyzing oxacillinases genes including *bla*$_{OXA-23}$, *bla*$_{OXA-24}$, and *bla*$_{OXA-58}$. Furthermore, *A. baumannii* strain KSU-DM24 and KSU-DM33, the most sensitive strains (10/17) had only one gene (*bla*$_{OXA-24}$), and none of the other screened genes, respectively, which could explain their high antibiotic susceptibility in comparison to other *A. baumannii* strains.

Conclusion

Clinical *A. baumanii* strains, isolated from patients hospitalized in various wards in King Khalid University and Armed Forces Hospitals, showed high level of resistance toward β-lactams antibiotics, including carbapenem antibiotics (imipenem and meropenem). Based on antibiotic susceptibility and in combination with the corresponding MIC values of each antibiotic, the isolated

Table 3. Distribution of various antibiotic resistance carbapenemases gene in the isolated clinical *A. baumannii* strains (n=40). P: Present; A: Absent.

A. baumannii strain	Carbapenemases genes		
	bla_{OXA-23}	bla_{OXA-24}	bla_{OXA-58}
KSU-DM1	P	A	P
KSU-DM2	A	A	P
KSU-DM3	P	P	P
KSU-DM4	P	A	A
KSU-DM5	P	A	P
KSU-DM6	P	P	P
KSU-DM7	P	P	P
KSU-DM8	A	P	P
KSU-DM9	P	P	P
KSU-DM10	P	P	A
KSU-DM11	A	P	A
KSU-DM12	A	P	A
KSU-DM13	P	P	A
KSU-DM14	P	P	A
KSU-DM15	P	A	A
KSU-DM16	A	A	A
KSU-DM17	P	P	A
KSU-DM18	P	A	A
KSU-DM19	P	A	A
KSU-DM20	P	A	A
KSU-DM21	P	A	P
KSU-DM22	P	P	A
KSU-DM23	P	P	P
KSU-DM24	A	P	A
KSU-DM25	A	A	A
KSU-DM26	P	A	P
KSU-DM27	P	P	A
KSU-DM28	A	P	P
KSU-DM29	P	P	P
KSU-DM30	P	P	A
KSU-DM31	P	A	A
KSU-DM32	P	A	A
KSU-DM33	A	A	A
KSU-DM34	A	A	A
KSU-DM35	A	A	A
KSU-DM36	P	A	A
KSU-DM37	P	A	P
KSU-DM38	P	A	P
KSU-DM39	P	A	A
KSU-DM40	P	A	A

A. baumannii strains was divided into four clusters (I, II, II and IV), where cluster I (n = 16) showing highest multiple antibiotic resistance (90 to 100% of the tested antibiotic and with very high MICs). Investigation of distribution of the major groups of carbapenem-hydro-lyzing oxacillinases genes in the isolated clinical *A. baumanii* strains revealed that the resistance to carba-penem antibiotics was due to bla_{OXA-23} followed by bla_{OXA-24}, and bla_{OXA-58} gene, respectively. In addition, analysis of the prevalence of different oxacillinases genes in different antibiotics-

Table 4. Distribution of various antibiotic resistance determinant genes (n=7) in different phenotypes of the isolated clinical *A. baumanni*i strains (n=40).

Phenotype	*bla*$_{OXA-23}$		*bla*$_{OXA-24}$		*bla*$_{OXA-58}$	
	No of isolates	%	No of isolates	%	No of isolates	%
Total (n=40)	29	72.5	18	45	15	37.5
Cluster 1 (n=16)	14	87.5	6	37.5	6	37.5
Cluster II (n=11)	9	81.8	4	36.4	2	18.2
Cluster III (n=9)	5	55.6	7	77.8	7	77.8
Cluster IV (n=4)	1	25	1	25	0	0

based phenotypes clusters, revealed that cluster I harbored the highest distribution of resistant genes, which could explain the extremely multiple antibiotic resistance phenotype within this cluster.

ACKNOWLEDGMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No RGP-VPP-045.

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