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ORIGINAL RESEARCH

Characterization of phenotypic and genotypic traits of carbapenem-resistant *Acinetobacter baumannii* clinical isolates recovered from a tertiary care hospital in Taif, Saudi Arabia

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Background and objective: *Acinetobacter baumannii* (*A. baumannii*) is a common nosocomial pathogen, which developed multi-drug-resistance to different classes of antibiotics including carbapenems. This study examined ten common carbapenemase genes among 32 carbapenem-resistant *A. baumannii* clinical isolates recovered from Taif, Saudi Arabia.

Methods: Isolates were phenotypically identified to the genus level by Vitek®2 and API 20NE®. The species level was confirmed by the amplification of $bla_{\text{OXA-51}}$. The susceptibility for 21 different antibiotics was performed by Vitek 2 and modified Kirby-Bauer method. Isolates were genetically screened for 10 carbapenemases. Phylogenetic relatedness between isolates was determined by ERIC-PCR.

Results: Genotypically identified *A. baumannii* represented 100% of the total phenotypically identified *Acinetobacter* spp. All the carbapenem-resistant isolates were sensitive to polymyxin B and colistin. Among the other antibiotics, ampicillin/sulbactam and tigecycline were the most effective agents. 90.8% of the isolates were resistant to all ten investigated β–lactams. $bla_{\text{OXA-51}}$, bla_{IPM}, bla_{NDM} and $bla_{\text{OXA-23}}$ were detected in 100%, 87.5%, 62.5% and 59.4% of isolates, respectively. Also, bla_{VIM} and $bla_{\text{OXA-40}}$ were less prevalent and were detected in 9.3% and 3.1% of the isolates, respectively. In addition, bla_{KPC}, $bla_{\text{OXA-48}}$, $bla_{\text{OXA-58}}$, $bla_{\text{OXA-181}}$ were not detected in any isolate. The *A. baumannii* isolates were categorised into ten genotypes on the basis of the detected carbapenemase genes and ERIC-PCR revealed a remarkable clonal diversity among these isolates.

Conclusion: Class A and class D carbapenemase genes were the most commonly detected among carbapenem resistant *A. baumannii* (CRAB) clinical isolates.

Keywords: *A. baumannii*, $bla_{\text{OXA-51}}$, carbapenemases, carbapenems, ERIC-PCR

Introduction

Acinetobacter spp. are recognized as important nosocomial pathogens. They cause a wide range of nosocomial infections including meningitis, urinary tract infection (UTI), bloodstream infection (BSI), wound infection (WI) and ventilator-associated pneumonia (VAP).[1] The genus *Acinetobacter* is strictly aerobic, non-motile, non-fermentative, oxidase-negative, and catalase-positive Gram-negative coccobacilli.[2–4]

The genus *Acinetobacter* includes more than 50 species. While some species are pathogenic, the majority are non-pathogenic.[5] The most common pathogenic species are *Acinetobacter baumannii* (*A. baumannii*) followed by *A. calcoaceticus* and

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A. lwoffii. Other occasionally reported pathogenic species include *A. haemolyticus* and *A. johnsonii*.[6] *A. baumannii* can be specifically identified on the molecular level by the detection of bla_{OXA-51}, which is absent in other species.[7]

A. baumannii is considered one of the most troublesome species due to its ability to resist a large number of antibiotics from different classes including carbapenem group.[8] In addition to its multi-drug- resistance (MDR), *A. baumannii* is associated with high morbidity and mortality in different hospital settings, especially the intensive care units (ICUs), where patients are immunocompromised.[2,9] According to the classification of Infectious Diseases Society of America (IDSA), *A. baumannii* is recognized as one of the six most important MDR microorganisms in hospitals worldwide.[10]

A. baumannii is considered MDR when it resists at least three classes of antimicrobial agents including all penicillins and cephalosporins, fluroquinolones, and aminoglycosides. On the other hand, it is considered an extensive drug resistant (XDR) when the MDR isolate is resistant to carbapenems. Pandrug resistant (PDR) *A. baumannii* is recognized when the XDR isolate is also resistant to polymyxins and tigecycline.[11]

Resistance to carbapenems is mediated by different mechanisms including (i) efflux pump, (ii) decreased membrane permeability through the loss of outer membrane porins (OMP) by downregulation of their synthesis and (iii) production of carbapenemases.[12]

The production of carbapenemases is one of the most important mechanisms responsible for carbapenem resistance among *A. baumannii* clinical isolates. According to Ambler classification of β-lactamases,[13] carbapenemases are related to three different molecular classes: (i) class A carbapenemases eg *Klebsiella pneumoniae* carbapenemase (KPC) and Imipenem-hydrolysing β-lactamase (IMI),[14] (ii) class B carbapenemases or the so-called metallo-β-lactamases eg Verona integrin metallo-β-lactamase (VIM), imipenemase (IMP) and New Delhi metallo-β-lactamases (NDM)[14–16] and (iii) class D carbapenemase or the so-called OXA-type carbapenemases eg OXA-23, OXA-40, OXA-48, OXA-51, OXA-58 and OXA-181.[17,18]

Patients and methods

Bacterial strains

The *A. baumannii* clinical isolates included in this study were collected from December 2017 to May 2017. They were isolated as a part of the routine hospital laboratory procedures and were further identified and confirmed in the laboratory. The study protocol was approved by Taif University Research Ethics Committee (approval 38-35-0021). Thirty-two non-duplicate non-consecutive clinical isolates of carbapenem-resistant *A. baumannii* were selected from a total of 45 *Acinetobacter* spp. clinical isolates. The isolates were recovered from 20 males and 12 females who were admitted to different medical departments at a large tertiary care hospital in Taif, KSA. The patients aged between 7 days to 97 years. The investigated isolates were recovered from different clinical specimens which included blood (n=12), tissue biopsy (n=1), peritoneal fluid (n=1), sputum (n=11), urine (n=2), wound (n=2), and catheter tip (n=3).

Isolation and identification

All strains were primarily isolated on blood agar and, then, purified on MacConkey's agar (Oxoid, UK). The genus and species level of the recovered *Acinetobacter* spp. isolates were primarily identified by Vitek®2 and API 20NE®. Molecular confirmation of *A. baumannii* clinical isolates was achieved through the amplification of the intrinsic bla_{OXA-51} gene by polymerase chain reaction (PCR).

Antimicrobial susceptibility testing (AST)

All recovered isolates were subjected to AST against 21 different antibiotics. The minimum inhibitory concentration (MIC) was determined for the available 13 antibiotics, in Vitek 2 GN ID card (Biomeriux, France), which are ceftazidime, cefotaxime, cefepime piperacillin/tazobactam, imipenem, meropenem, gentamicin, amikacin, tobramycin, ciprofloxacin, levofloxacin, tetracycline and sulfamethoxazole/trimethoprim. Modified Kirby-Bauer method was used to confirm the results of the Vitek 2 system and to test the susceptibility to ticarcillin, piperacillin, ampicillin/sulbactam, ceftriaxone, netilmicin, polymyxin B, colistin (Biorad, USA) and tigecycline (Wyeth, USA), that are not available in the Vitek® system. *Escherichia coli* (*E. coli*) ATCC 25,922 and *Klebsiella pneumonia* (*K. pneumoniae*) ATCC 700,603 were used as quality control standard strains. Results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[19]

Preparation of DNA templates

The boiling method was applied for total genomic DNA extraction as previously described.[20] Briefly, three to six colonies of the bacterial isolates (depending on colony size) were picked from a tryptic soy agar (Scharlau,

Spain) plate and suspended in 100µl of DNase-free water in a sterile 1.5ml microfuge tube to obtain a bacterial suspension equivalent to 1-2×10^9 CFU/mL. The bacterial suspension was placed in a boiling water bath for 10 min. The lysed suspension was centrifuged at a speed of 13200rpm for 5 mins. The supernatant containing the total genomic DNA extract was transferred to a new sterile DNase free microfuge tube using DNase-free tips. The total genomic DNA extract was stored at −20°C until used.

Genotypic detection of carbapenemases

All carbapenem-resistant isolates were screened for 10 carbapenemase encoding genes; Two class A carbapenemases (bla_{IPM} and bla_{KPC}), two class B carbapenemases (bla_{NDM}, and bla_{VIM}) and six class D carbapenemase (bla_{OXA-23}, bla_{OXA-40}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58} and $bla_{OXA-181}$). Target gene amplification was performed using primers (Geumcheon-gu, Seoul, Korea) and the cycling conditions listed in Table 1.

PCR

The PCR reaction was performed in a final reaction volume of 40µl. The reaction mixture contained 8 µl of the extracted DNA, 8 µl of 5x master mix (HOT FIREPol® Blend Master Mix, Solis BioDyne, Tartu, Estonia), 1.2 µl of the forward primer (10 pmol/µl), 1.2 µl of the reverse primer (10 pmol/µl) and 21.6 µl sterile distilled water. The 0.2 PCR tubes that contained the reaction mixture were placed in the thermal cycler and the reaction was processed for each gene according to cyclic conditions listed in Table 1.

Genotyping of the clinical isolates

Clonal relatedness between *A. baumannii* clinical isolates was determined by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR). Amplification of the repeated intergenic consensus regions was performed using Mastercycler® personal (Eppendorf, California, USA), and the primers and cycling conditions described in Table 1. The amplified fragments were run on a 2.5% agarose at 100 volt for 90 mins. The agarose gel was removed from the gel tank and placed on a UV- transilluminator tray in which images were captured.

Fingerprint pattern analysis

The generated ERIC-PCR profiles were analysed by BioNumerics 7.5® software (Applied Maths, Kortrijk, Belgium) as previously described.[21] The captured gel images stained with ethidum bromide were uploaded to the software and were analysed to generate the dendrogram.

The generated dendrogram was performed according to the Dice similarity coefficient based on the unweighted pair group method with arithmetic averages (UPGMA) at position tolerance at 0.15.

Results

Isolation and phenotypic identification

According to the identification by Vitek 2 system, all isolates were related to the genus *Acinetobacter*, in which 56.3% (18/32) were *A. baumannii/haemolyticus*, 37.5% (12/32) were *A. baumannii complex/haemolyticus* and 6.3% (2/32) were *A. lwoffii*. On the other hand, phenotypic identification by API 20NE revealed that all isolates were related to the genus *Acinetobacter* with different levels of possible identification in which 50% (16/32) of the isolates were *A. baumannii/calcoaceticus* with excellent identification (98.8%), 40.6% (13/3) were *A. baumannii/calcoaceticus* with low discrimination (66.5%) and 9.4% (3/32) were *A. baumannii/ calcoaceticus* with low discrimination (26.5%).

Molecular confirmation of A. baumannii

Molecular investigation of bla_{OXA-51} revealed that 100% of the phenotypically identified *Acinetobacter* spp. isolates were positive, so they were considered as *A. baumannii.*

Antimicrobial susceptibility

Antimicrobial susceptibility testing revealed that polymyxin B and colistin were the most effective agents as all isolates were sensitive to both agents. On the other hand, both tigecycline and ampicillin/sulbactam were ranked as the second effective agents since 75% (24/32) and 62.5% (20/32) of the isolates were sensitive, respectively. It should be mentioned that twelve isolates (37.5%) were intermediately resistant to ampicillin/sulbactam and none of the isolates was resistant to that combination. The isolates had high resistance rates to both 3rd and 4th generation cephalosporins in which at least 90.8% (29/32) of the isolates were resistant.

With regard to the two tested quinolone antibiotics, 100% (32/32) and 78.1% (25/32)of isolates were resistant to ciprofloxacin and levofloxacin, respectively. The resistance rate to aminoglycosides ranged between 56.3 (18/32) and 90.6% (29/32). Lower resistance rates of 56.3% (18/32) and 59.4% (19/32) were observed for gentamicin and tobramycin, respectively as shown in Table 2.

All *A. baumannii* isolates had multiple drug-resistance to at least 13 of the 21 tested antibiotics (Table 3). The

Table 1 Primers and cycling conditions used for the amplification of carbapenemase genes and the repetitive intergenic consensus

Primer	Sequence	Gene	Reference	Amplification conditions	Amplicon size (bp)
ERIC-1R	**R:**AACCCACGATGTGGGTAGC	-	50	**Initial denaturation** at 95°C for 15 min, then **35 cycles of** 95°C for 1 min, 40°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72°C.	-
IPM	**F:** GGAATAGRRTGGCTTAAYT **R:** GGTTTAAYAAARCAMCCACC	***bla*$_{IPM}$**	51	**Initial denaturation** at 95°C for 15 min, then **35 cycles of** 95°C for 1 min, 40°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72°C.	233
KPC	**F:** GTATCGCCGTCTAGTTCTGC **R:** GGTCGTGTTTCCCTTTAGCC	***bla*$_{KPC}$**	52	**Initial denaturation** at 95°C for 15 min, then **30 cycles of** 95°C for 1 min, 55°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72°C.	637
NDM	**F:** GGTTTGGCGATCTGGTTTTC **R:** CGGAATGGCTCATCACGATC	***bla*$_{NDM}$**	53	**Initial denaturation** at 95°C for 15 min, then **30 cycles of** 95°C for 1 min, 66.7°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72°C.	621
VIM	**F:** GTTTGGTCGCATATCGCAAC **R:** GAGCAAKTCYAGACCGCCC	***bla*$_{VIM}$**	51	**Initial denaturation** at 95°C for 15 min, then **30 cycles of** 95°C for 1 min, 63°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72°C.	591
OXA-23	**F:** GATCGGATTGGAGAACCAGA **R:** ATTTCTGACCGCATTTCCAT	***bla*$_{OXA-23}$**	54	**Initial denaturation** at 95°C for 15 min, then **30 cycles of** 95°C for 1 min, 66.7°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72°C.	501
OXA-40	**F:** GGTTAGTTGGCCCCCTTAAA **R:** AGTTGAGCGAAAAGGGGATT	***bla*$_{OXA-40}$**	55	**Initial denaturation** at 95°C for 15 min, then **30 cycles of** 95°C for 1 min, 66.6°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72°C.	249
OXA-48	**F:** GCTTGATCGCCCTCGATT **R:** GATTTGCTCCGTGGCCGAAA	***bla*$_{OXA-48}$**	56	**Initial denaturation** at 95°C for 15 min, then **30 cycles of** 95°C for 1 min, 60.5°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72°C.	281
OXA-51	**F:** TAATGCTTTGATCGGCCTTG **R:** TGGATTGCACTTCATCTTGG	***bla*$_{OXA-51}$**	57	**Initial denaturation** at 95°C for 15 min, then **30 cycles of** 95°C for 1 min, 60.5°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72 °C.	353
OXA-58	**F:** AAGTATTGGGGCTTGTGCTG R: CCCCTCTGCGCTCTACATAC	***bla*$_{OXA-58}$**	54	**Initial denaturation** at 95°C for 15 min, then **30 cycles of** 95°C for 1 min, 60.5°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72°C.	599
OXA-181	**F:** ATGCGTGTATTAGCCTTATCG R: AACTACAAGCGCATCGAGCA	***bla*$_{OXA-181}$**	22	**Initial denaturation** at 95°C for 15 min, then **30 cycles of** 95°C for 1 min, 55°C for 1min and 72°C for 5 minutes and **one cycle** of **final elongation** at 72°C.	888

Notes: R= (A⁄G), Y= (C⁄ T), K= (G⁄ T) and M= (A⁄C). Boldface in this table clarifies primers and different stages of the PCR cycles.

percentage of isolates resistant to 17–19 antibiotics was 59.4% (19/32). On the other hand, the isolates were resistant to at least eight β-lactams and 12/32 (37.7%) were resistant to the ten tested β-lactams.

Genotypic detection of carbapenemases

The investigation of class A carbapenemases revealed that 87.5% (28/32) of the isolates were positive for bla_{IPM} gene while none of the isolates harboured the bla_{KPC} gene. With regard to class B carbapenemases (metallo-carbapenemase), it was found that 62.5% (20/32) and 9.4% (3/32) of the isolates harboured bla_{NDM} and bla_{VIM}, respectively (Table 4).

Class D carbapenemases (OXA-type carbapenemases) were the most prevalent among CRAB isolates where 59.4% (19/32), 3.1% (1/32) and 100% (32/32) of the isolates were positive for bla_{OXA-23}, bla_{OXA-40} and bla_{OXA-51} genes, respectively. On the other hand, bla_{OXA-48} and bla_{OXA-58} and $bla_{OXA-181}$ genes were not detected.

Analysis of carbapenemase genetic profiles of the 10 investigated carbapenemase genes revealed nine different genetic profiles (A-I). The most common profile was profile B, which was detected in 25% (8/32) of the isolates followed by the A and J profiles; each was detected in 18.8% (6/32) of the isolates (Table 4). The least detected genetic carbapenemase profiles were C, E, G, H and I that were detected in only one isolate each.

Fingerprint pattern analysis

The DNA fingerprint patterns of *A. baumannii* isolates were generated by ERIC-PCR as shown in Figure 1. The generated dendrogram at 80% similarity demonstrated 28 different fingerprint profiles with high clonal variability. Twenty-five isolates had 25 different profiles and 7 isolates had the remaining three profiles. The three profiles included one single profile for AC19, AC26 and AC32, one single profile for AC27 and AC29 isolates and one single profile for AC2 and AC3 isolates (Figure 2). The generated UPGMA dendrogram categorized the 32 isolates into two main phylogenetic groups (A and B). While phylogenetic group A (PGA) included only one isolate

Table 2 Susceptibility pattern of *A. baumannii* clinical isolates

Antibiotic	Susceptibility pattern					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Cefotaxime	0.00	0.00	1.00	3.12	31.00	96.88
Ceftazidime	2.00	6.24	0.00	0.00	30.00	93.76
Ceftriaxone	1.00	3.12	1.00	3.12	30.00	93.76
Cefepime	0.00	0.00	3.00	9.36	29.00	90.64
Imipenem	0.00	0.00	0.00	0.00	32.00	100.00
Meropenem	0.00	0.00	2.00	6.24	30.00	93.76
Ticarcillin	0.00	0.00	0.00	0.00	32.00	100.00
Piperacillin	0.00	0.00	0.00	0.00	32.00	100.00
Piperacillin/Tazobactam	0.00	0.00	0.00	0.00	32.00	100.00
Ampicillin/Sulbactam	20.00	62.50	12.00	37.50	0.00	0.00
Gentamicin	5.00	15.63	9.00	28.13	18.00	56.25
Tobramycin	11.00	34.38	2.00	6.24	19.00	59.38
Amikacin	3.00	9.36	0.00	0.00	29.00	90.64
Netilmicin	8.00	25.00	0.00	0.00	25.00	75.00
Ciprofloxacin	0.00	0.00	0.00	0.00	32.00	100.00
Levofloxacin	0.00	0.00	7.00	21.88	25.00	78.12
Tetracycline	3.00	9.36	0.00	0.00	29.00	90.64
Tigecycline	24.00	75.00	0.00	0.00	9.00	25.00
Sulfamethoxazole/Trimethoprim	6.00	18.75	0.00	0.00	26.00	81.25
Polymyxin B	32.00	100.00	0.00	0.00	0.00	0.00
Colistin	32.00	100.00	0.00	0.00	0.00	0.00

Table 3 Resistance patterns of the *A. baumannii* clinical isolates

Isolate No.	Antibiotic resistance profile	Resistance markers
AC01	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, NET, TET, CIP, LEV, SXT	18
AC02	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, TOB, AK, NET, TET, CIP, LEV, SXT	16
AC03	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, AK, TET, CIP, LEV, SXT	13
AC04	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, NET, TET, CIP, LEV, TEG, SXT	19
AC05	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, TET, CIP, LEV, SXT	13
AC06	CTX, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, AK, NET, CIP, LEV	13
AC07	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, TOB, AK, NET, TET, CIP, LEV, SXT	17
AC08	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, TOB, AK, NET, TET, CIP, LEV, SXT	17
AC66	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, AK, NET, TET, CIP, LEV, SXT	15
AC10	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, TOB, CIP, LEV	13
AC11	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, NET, TET, CIP, LEV, TEG, SXT	19
AC12	CTX, CAZ, FEP, IPM, MEM, TIC, PIP, TPZ, CN, AK, TET, CIP, LEV, SXT	14
AC13	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, NET, TET, CIP, LEV, SXT	18
AC14	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, TOB, AK, NET, TET, CIP, LEV, SXT	17
AC15	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, TOB, AK, NET, TET, CIP, LEV	16
AC16	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, TOB, AK, NET, TET, CIP, LEV, SXT	17
AC17	CTX, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, AK, NET, CIP, LEV	13
AC18	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, TET, CIP, LEV, TEG, SXT	18
AC19	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, AK, NET, TET, CIP, LEV, SXT	15
AC20	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, AK, NET, TET, CIP, LEV, SXT	16
AC21	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, AK, NET, TET, CIP, LEV, SXT	16
AC22	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, NET, TET, CIP, LEV, TEG, SXT	19
AC23	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, NET, TET, CIP, LEV, TEG, SXT	19
AC24	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, TOB, AK, NET, TET, CIP, LEV, TEG	17
AC25	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, TOB, AK, NET, TET, CIP, LEV, TEG	17
AC26	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, CN, TOB, AK, NET, TET, CIP, LEV, SXT	17
AC27	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, AK, NET, TET, CIP, LEV, SXT	17
AC28	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, NET, TET, CIP, LEV, SXT	18
AC29	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, NET, TET, CIP, LEV, TEG, SXT	19
AC30	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, TET, CIP, LEV, SXT	13
AC31	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, NET, TET, CIP, LEV, SXT	18
AC32	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM, CN, TOB, AK, NET, TET, CIP, LEV, SXT	18

Abbreviations: CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; IPM, imipenem; MEM, meropenem; TIC, ticarcillin; PIP, piperacillin; TPZ, piperacillin/tazobactam; SAM, ampicillin/sulbactam; CN, gentamicin; TOB, tobramycin; NET, netilmicin; CIP, ciprofloxacin; LEV, levofloxacin; TET, tetracycline; TEG, tigecycline; SXT, sulfamethoxazole/trimethoprim.

(AC9), phylogenetic group B (PGB) hosted 31 isolates (96.9%). PGB was further subdivided into two main sub-phylogenetic groups (PGB.1 and PGB.2). As shown in Figure 2, PGB 2 was further sub-classified into three main clades B.2.1, B.2.2.1 and B.2.2.2. Subgroup B2.2.1 represented 43.8% (14/32) of the investigated isolates.

Discussion

A. baumannii is one of the most important opportunistic pathogens that cause hospital-acquired epidemics.[2,10] Patients with complicated infections by *A. baumannii* have been treated with carbapenems as drugs of choice to save their lives. However, the number of CRAB has been increasing in the KSA and worldwide.[22] Fortunately, recently approved antibiotics and antibiotic/inhibitor combinations such as ceftazidime/avibactam, meropenem/vaborbactam, ceftolozane/tazobactam, plazomicin, and eravacycline are effective in the treatment of infections caused by CRAB.[23]

In the present study, 32 molecularly confirmed CRAB clinical isolates were chosen out of 45 phenotypically identified *Acinetobacter* spp. isolates to investigate (i) antimicrobial susceptibility to 21 different antibiotics; (ii) the rate of harbouring 10 carbapenemase genes and (iii) the clonal relationship between the investigated resistant strains. To our knowledge from the available literature, this is the first report from Taif area.

All the investigated CRAB isolates were MDR. The isolates were resistant to 13–19 of the 21 investigated antibiotics.

Table 4 Relationship between genotypic profiles of carbapenemases and phenotypic resistance profiles *A. baumannii* for β-lactam antibiotics

No	Genetic Profile	No of isolates	Profiles of detected β -lactamase genes	Phenotypic resistance profiles to β -lactams	Resistance markers
1	A	6	$bla_{OXA\text{-}51}$, bla_{IPM}, bla_{NDM}	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM	9-10
2	B	8	$bla_{OXA\text{-}51}$, $bla_{OXA\text{-}23}$, bla_{IPM}	CTX, CRO, FEP, IPM, MEM, TIC, PIP, TPZ CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM	8-10
3	C	1	$bla_{OXA\text{-}51}$, $bla_{OXA\text{-}23}$	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM	10
4	D	6	$bla_{OXA\text{-}51}$	CTX, CAZ, FEP, IPM, MEM, TIC, PIP, TPZ CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM	8-10
5	E	1	$bla_{OXA\text{-}51}$, $bla_{OXA\text{-}40}$	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ	9
6	F	2	$bla_{OXA\text{-}51}$, $bla_{OXA\text{-}23}$, bla_{IPM}, bla_{NDM}, bla_{VIM}	CTX, CRO, FEP, IPM, MEM, TIC, PIP, TPZ CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ	8-9
7	G	1	$bla_{OXA\text{-}51}$, $bla_{OXA\text{-}23}$, bla_{IPM}, bla_{VIM}	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM	10
8	H	1	$bla_{OXA\text{-}51}$, $bla_{OXA\text{-}23}$, bla_{NDM}	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ	9
9	I	6	$bla_{OXA\text{-}51}$, $bla_{OXA\text{-}23}$, bla_{IPM}, bla_{NDM}	CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ CTX, CAZ, CRO, FEP, IPM, MEM, TIC, PIP, TPZ, SAM	9-10

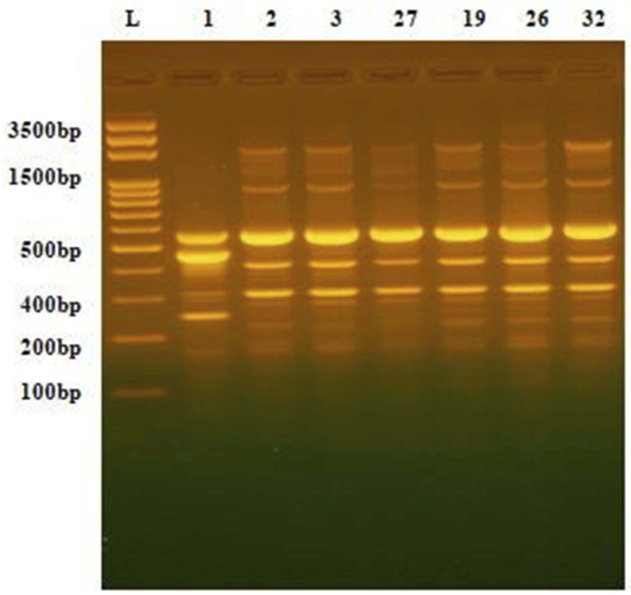

Figure 1 DNA fingerprint pattern generated by ERIC-PCR for *Acinetobacter* clinical isolates. L, 100bp DNA ladder; lanes 1, 2, 3, 27,19, 26 and 32 represent the code No. of the isolates.

Apart from ampicillin/sulbactam combination, for which none of the isolates was resistant, at least 90.8% of the isolates were resistant to all the ten investigated β–lactams.

All the isolates were sensitive to polymyxin B and colistin. Therefore, these antibiotics remain the best empirical therapeutic choices for the treatment of infection caused by CRAB in high-risk patients in Taif. The previous findings are in line with a recent report that suggests that polymyxins are often the last line of treatment for recalcitrant infections by CRAB isolates.[24] Nonetheless, it should be mentioned that isolates resistant to both carbapenems and colistin have been reported elsewhere in the Kingdome of Saudi Arabia.[25–28]

While, the resistance rates to aminoglycosides, quinolones and tetracycline ranged between 56.3 to 100%, only 25% of the isolates were resistant to tigecycline. Higher rates of resistance to tigecycline have been reported in other areas of KSA.[29–31]

In spite of the fact that carbapenemases can't be inhibited by serine class A β–lactamases inhibitors,[32,33] it was found that 62.5% of the isolates were sensitive to ampicillin/sulbactam and 37.5% were intermediately resistant. The antibacterial effect of ampicillin/sulbactam against the carbapenem-resistant *A. baumannii* is probably due to the antibacterial activity exerted by sulbactam against *A. baumannii*, rather than the inhibition of their carbapenemases. In a previous study, sulbactam was used successfully to treat 14 patients with VAP caused by MDR *Acinetobacter* spp.[34]

The significant prevalence of MDR in nosocomial infections caused by *A. baumannii* along with the rapid development of XDR and PDR limits the therapeutic options for the treatment of serious infections caused by *A. baumannii*.[35,36] Herein, the rate of XDR among our isolates was 71.1% (32/45). Fortunately, none of the isolates exhibited resistance to both tigecycline and polymyxin. Therefore, according to the criteria proposed by Manchanda, et al, none of the isolates was categorised as PDR.[11]

In this study, all isolates harboured bla_{OXA51} either alone or in combination with either $bla_{\text{OXA-40}}$, or $bla_{\text{OXA-23}}$. In this regard, $bla_{\text{OXA-51}}$ is located on the chromosome of *A. baumannii*[7] and therefore, such a gene has been utilized for molecular confirmation of *A. baumannii*. $bla_{\text{OXA-40}}$ was detected in only one isolate (3.1%), which is lower than its incidence reported by others across other regions in KSA like Aseer and the Eastern region, in which the incidence of $bla_{\text{OXA-40}}$ in such regions ranged between 13 to 30%.[25,37,38] On the other hand, $bla_{\text{OXA-23}}$ was detected in 19 isolates (59.4%). This resistance gene has been reported as one of the most detected carbapenemases in Saudi Arabia and the Gulf area.[9,39] The incidence of $bla_{\text{OXA-23}}$ in KSA is controversial. For instance, similar[26,28,38] and higher rates (16–18) have been reported in the Eastern region and Riyadh. The $bla_{\text{OXA-23}}$ gene is plasmid-encoded.[33] Therefore, the horizontal dissemination of harbouring plasmids may explain the widespread of $bla_{\text{OXA-23}}$ in Taif and other parts of the KSA. One of the isolates harboured $bla_{\text{OXA-40}}$ while none of the isolates harboured the $bla_{\text{OXA-48}}$, $bla_{\text{OXA-58}}$ and $bla_{\text{OXA-181}}$ genes. $bla_{\text{OXA-58}}$ was previously reported in Aseer and Riyadh at low incidences of 3.6%[26] and 1.6%,[25] respectively. On the other hand, while $bla_{\text{OXA-40}}$ was prevalent in 30% of *A. baumannii* isolated from diabetic patients,[37] $bla_{\text{OXA-181}}$ and $bla_{\text{OXA-48}}$ have not been reported in KSA.[40]

The current study revealed that 20 (62.5%) of CRAB isolates harboured two bla_{OXA} genes. One isolate (3.1%) harboured $bla_{\text{OXA-40}}$ and 19 isolates (59.4%) harboured both bla_{OXA51} and $bla_{\text{OXA-23}}$. The presence of more than one type of bla_{OXA} genes is common in the *A. baumannii* in KSA.[40]

The investigated isolates were screened for three metallo-carbapenemase genes, namely, NDM, VIM and IMP. Metallo-β-lactamases are not inhibited by β-lactamase inhibitors, which are active against serine-based, class A β–lactamases like clavulanate, sulbactam, or tazobactam.[33,41] No clinically available inhibitors are currently available to block metallo-β-lactamases. Out of the 32 investigated *A. baumannii* isolates, 24 (75%) harboured one to three

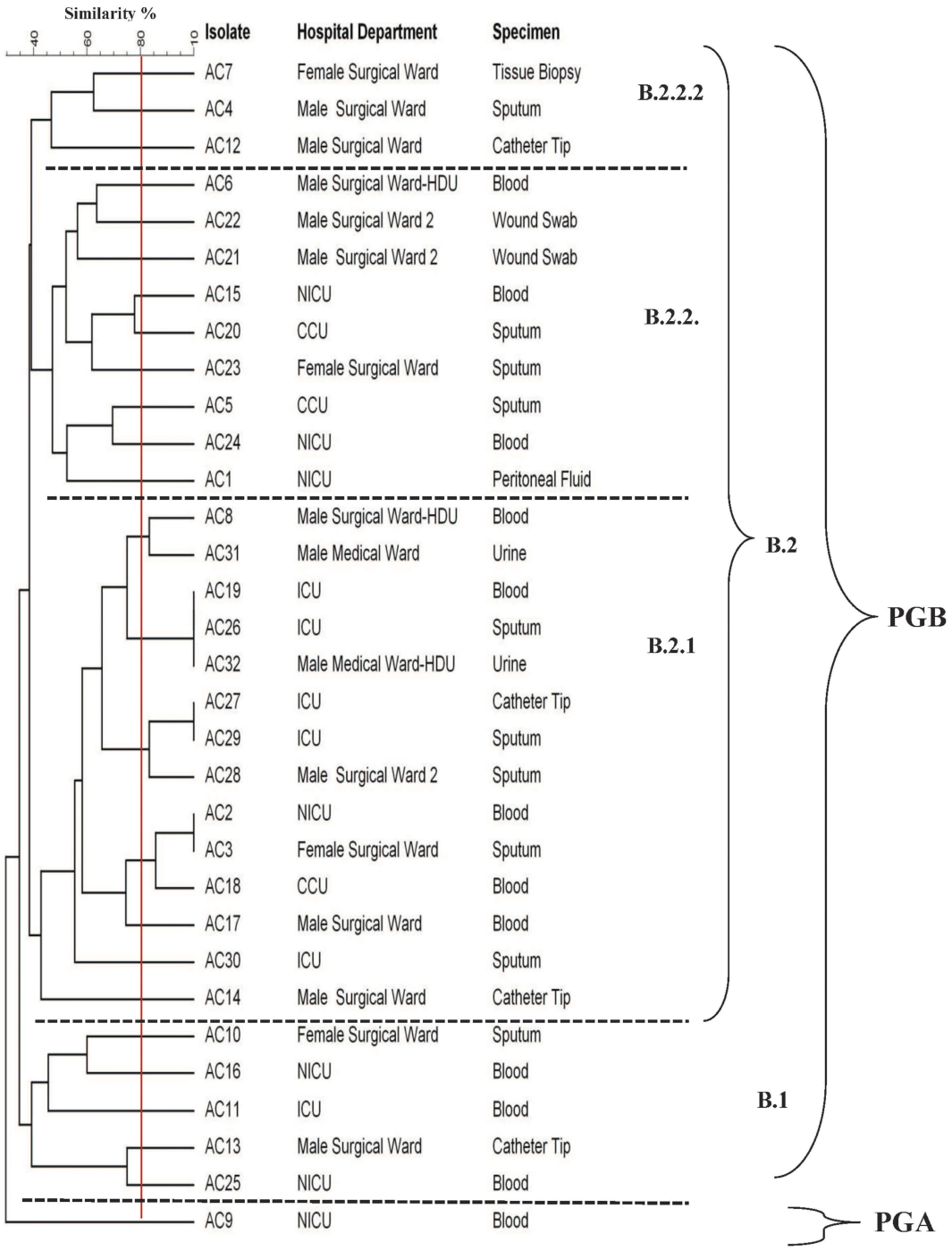

Figure 2 Clonal relationship between *A. baumannii* clinical isolates generated by UPGMA dendrogram.

metalloenzymes. The genes of bla_{NDM}, bla_{IPM}, bla_{VIM} were incident in 46.9%, 71.9%, and 9.4%, of the isolates, respectively.

To our knowledge from the literature, bla_{NDM} was first detected in 2008 in *K. pneumonia* and *E. coli* in a patient returning to Sweden from India.[42] In this study, bla_{NDM} was detected in 62.5% of the isolates. Based on the previous finding, we conclude that bla_{NDM} was probably introduced to KSA through the large number of Indian labours working in the Kingdom. bla_{NDM} has been found to be located on several types of plasmids.[43] The location of bla_{NDM} on the plasmids contributes to its rapid horizontal spreading among Gram-negative bacteria by conjugation.[44] In this study, bla_{NDM} was detected in 9.4% of the isolates. Lower rates (2.4%) and higher rates (30%), for bla_{NDM}, were reported among *A. baumannii*

collected across the KSA[38] and in the Eastern District of the KSA, respectively.[31]

The incidences of bla_{IMP} and bla_{VIM} in this study were 71.9% and 9.4%, respectively. Both bla_{IMP} and bla_{VIM} reside with other resistance genes on integrons associated with transposons. This facilitates their translocation between the chromosome and plasmids[33] and their rapid horizontal dissemination.[45]

The present study revealed that two to five class B and D carbapenemases co-existed in 81.3% of the investigated CRAB clinical isolates. The presence of multiple genes responsible for resistance to carbapenems has been well documented in the KSA.[28,39,40,46]

The data on the clonal relationship between the *A. baumannii* isolates reflects their diversity in society and helps in choosing and designing some preventive methods to limit their spreading. ERIC-PCR was performed in this study because it is a rapid and reliable method for studying the phylogenetic relationship between the isolates.[47] The ERIC-PCR data in this study shed the light on the remarkable clonal diversity among CRAB clinical isolates (28 clones among 32 strains) collected from a tertiary care hospital in Taif, KSA rather than the predominance of certain epidemic clones. This suggests the circulation of different *A. baumannii* clones in the hospital. The strains possessing the same ERIC-PCR pattern did not necessarily exhibit the same antibiotic resistance pattern or harbour the same resistance genes. This may be attributed to the process of horizontal resistance gene transfer between bacteria under the high selection pressure in the hospital.

Annually, more than 1.5 million Muslims from across the globe gather in Makkah to perform the rites of pilgrimage and Umrah. The mass gatherings in the Holy City provide a good environment for exchanging microorganisms and the dissemination of various antibiotic resistance genes between the visitors and the Makkans.[42] French Muslim pilgrims were screened for the acquired bacteria during Hajj. One of the acquired isolates of *A. baumannii* harboured a bla_{OXA-72},[36] which is rarely detected in KSA.[39,40] The location of Taif near Makkah and the continuous mixing of the population of both cities might be one of the factors involved in the high rate of MDR observed in *A. baumannii* isolates collected from Taif.

In conclusion, this study exposes the problem of carbapenem-resistance in Taif area. Data obtained herein emphasize the necessity of the application of strict infection control measures.[48,49] Generally, there should be protocols for screening high-risk patients for carbapenem-resistant pathogens before their admission to healthcare facilities. Also, stewardship guidelines are recommended to restrict the irrational use of antibiotics in healthcare facilities, community pharmacies and agricultural settings.

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Disclosure

The authors report no conflicts of interest in this work.

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