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Molecular detection of oxacillinase genes and typing of clinical isolates of *Acinetobacter baumannii* in Tehran, Iran

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ABSTRACT

Objective: To determine patterns of antimicrobial resistance, analyze the prevalence of oxacillinase and molecular typing of strains of *Acinetobacter baumannii* (*A. baumannii*).

Methods: A total of 121 strains of *A. baumannii* were obtained from patients admitted to Imam Hossein and Imam Khomeini Hospitals, Tehran, Iran, from January 2016 to November 2018. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute recommendations. The presence of oxacillinase genes was assessed by polymerase chain reaction (PCR). To determine clonal relatedness, all isolates were subjected to repetitive sequence-based PCR (REP-PCR).

Results: The isolates were obtained from 56 (46.3%) males and 65 (53.7%) females with the mean age of 39.5 years. Colistin with 100.0% sensitivity rate had the highest effect, while ceftriaxone with 16.5% sensitivity rate had the least effect on *A. baumannii* isolates. In addition, 96 (79.3%) and 99 (81.8%) isolates were resistant to imipenem and meropenem, respectively. A total of 109 isolates (90.0%) exhibited multiple drug resistance with 10 different resistotypes. In total, 75 (75.7%) of carbapenem resistant isolates were positive for $bla_{\text{OXA-23-like}}$, and 14 (14.1%) for $bla_{\text{OXA-24-like}}$ gene. The five main clones A, B, C, D, and E were detected in 25 (25.2%), 36 (36.4%), 10 (10.1%), 8 (8.0%), and 6 (6.1%) of isolates, respectively.

Conclusions: Carbapenem-resistant *A. baumannii* strains are high in the current study. To control the spread of carbapenem-resistant *A. baumannii* strains, regular monitoring programs are needed.

KEYWORDS: *Acinetobacter baumannii*; Multidrug resistance; Oxacillinase; Repetitive sequence-based PCR

1. Introduction

Acinetobacter baumannii (*A. baumannii*), an opportunistic Gram-negative, aerobic, non-fermenting cocobacilli, is mostly found in many healthcare environments, different water sources and soil. This bacterium is ample in mucous membranes and normal flora of skin in humans and able to cause different infections such as septicemia, meningitis, urinary tract infection, upper respiratory tract infections, pneumonia and especially in intensive care units (ICUs) worldwide[1].

A. baumannii is resistant to a number of commonly used antibacterial drugs intrinsically, and has a significant capacity to develop antibiotic resistance *via* different mechanisms[2]. Plasmids, integrons and transposones are the most important interchangeable genetic agents that play an important role in the transfer of antibiotic resistance genes. Resistance to various antibiotics such as carbapenems, fluoroquinolones, ami noglycosides and broad spectrum β-lactams are observed in this bacterium, which complicates the treatment of infections. Nowadays, *A. baumannii* became more widespread as an important pathogen, specifically as a multidrug-resistant agent (MDR)[3,4].

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Therapeutic issues caused by MDR *A. baumannii* and long-lasting nature in hospital environments and also the ability to transfer between living and non-living things has caused enhancment in the appearance of the bacteria and its increasing infection. Thus, mortality in infections caused by *A. baumannii* is estimated to be about 75%[5].

Resistance to carbapenem is now observed globally in *A. baumannii* and these isolates are typically resistant to various classes of antibacterial agents. Several outbreaks caused by carbapenem resistant among *A. baumannii* isolates are reported from different countries of the world which could be an alarm for global health[6]. *A. baumannii* has different mechanisms for resistance to carbapenems, which include changes in penicillin binding protein, efflux pumps and mostly by production of oxacillinases (OXAs) and less common by metallo β-lactamase (MBLs) genes, AmpC stable derepression and reduced permeability[7,8].

The first report of OXA-type beta-lactamases in *A. baumannii* was related to *Acinetobacter* resistant to imipenem (ARI-1) that later designated as OXA-23[4]. Today, OXA-type carbapenemases have eight subgroups, four of which in *A. baumannii* have been reported: OXA-23-like consisting of OXA-23, OXA-27 and OXA-49; OXA-24-like (OXA-24, OXA-25, OXA-26, OXA-40 and OXA-72); OXA-51-like and OXA-58[9]. The transmission of isolates resistant to carbapenem *A. baumannii* is shown in the hospital. For control of resistant isolates and epidemiological aims, fast and accurate differentiation of epidemic strains from the many incidental strains is important[10,11].

One of the molecular typing methods used to determine the clonal relatedness of *A. baumannii* is repetitive sequence-based polymerase chain reaction (REP-PCR)[12]. The aim of this study was to survey the antibiotic susceptibility, prevalence of oxacillinase genes and perform molecular typing by REP-PCR in *A. baumannii* clinical isolates.

2. Materials and methods

2.1. Ethics statement and informed consent

This research was confirmed by the Ethical Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran with ethics number: IR.SBMU.RETECH.REC.1397.464. Informed consent form was obtained from all of the patients.

2.2. Study design and specimens collection

This cross-sectional study was performed by using 121 *A. baumannii* strains isolated from patients admitted to Imam Hossein and Imam Khomeini hospitals located in Tehran, Iran, from January 2016 to November 2018. A questionnaire was designed and coded for each patient. The samples were taken from patients included blood, trachea, urine, cerebrospinal fluid, catheter and pleural fluid.

These samples were cultured on blood agar and MacConkey (Merck,Germany) to confirm the Gram-negative bacteria *via* Gram stain microscopy analysis. Bacterial isolates were initially identified as *A. baumannii* by biochemical tests [Oxidase, nitrate reduction, motility on sulfide indole motility, growth at 42℃ and on MacConkey agar, oxidative-fermentative glucose, sugars fermentation on triple sugar iron agar, citrate utilization on Simon's Citrate agar]. Also, the final confirmation of *A. baumannii* isolates was conducted by PCR of $bla_{\text{OXA-51-like}}$ gene[13,14].

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI 2017) guidelines using the following antibiotics: amikacin (30 μg), imipenem (10 μg), meropenem (10 μg), piperacillin-tazobactam (10/100 μg), cefepime (30 μg), ceftriaxone (30 μg), tetracycline (10 μg), tigecycline (15 μg), ciprofloxacing(5 μg), tobramycin (10 μg) and colistin (10 μg) (MAST, UK)[15]. *A. baumannii* ATCC19606 was used as the control strain, and Pseudomonas aeruginosa ATCC 27853 and *Escherichia coli* ATCC 25922 were used as negative controls.

2.4. PCR amplification and detection of OXA genes

PCR was conducted to detect $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-143-like}}$. All target genes and corresponding primers used for PCR amplification are shown in Table 1[16]. Bacterial DNA was obtained by boiling method[17]. The PCR mixture contained master mix (30 Mm of KCL: 10 Mm, 30 Mm, 10 Mm of Tris-HCL, Bioneer Company, Korea), the forward/reverse primers and DNA template. PCR conditions are included 30 cycles of amplification under the following conditions in Mastercycler Eppendorf (Eppendorf, Germany): denaturation at 95℃ for 5 min; annealing at 55℃ for 30 sec; and 45 sec for extension at 72℃, with a final extension at 72℃ for 6 min.

Table 1. Primers used to detect genes encoding oxacillinases[16].

Primer	Sequence (5'- 3')	Product size (bp)
OXA–23	F-GATCGGATTGGAGAACCAGA	501
	R-ATTTCTGACCGCATTTCCAT	
OXA–24	F-GGTTAGTTGGCCCCCTTAAA	246
	R-AGTTGAGCGAAAAGGGGATT	
OXA–51	F-TAATGCTTTGATCGGCCTTG	353
	R-TGGATTGCACTTCATCTTGG	
OXA–58	F-AAGTATTGGGGCTTGTGCTG	599
	R-CCCCTCTGCGCTCTACATAC	
OXA–143	F-TGGCACTTTCAGCAGTTCCT	149
	R-TAATCTTGAGGGGGCCAACC	

2.5. REP–PCR

Genetic relationship of all isolates was determined by REP-PCR. In the REP-PCR method, the primer pair of REP-F (REP-Ⅰ, Ⅲ:GCGCCGICATCAGGC) and REP-R (REP-Ⅱ: ACGTCTTATCAGGCCTAC) was used and amplification PCRs were performed as previously described[18]. The amplified products were separated via electrophoresis on 1.5% agarose gels and compared by visual inspection. The amplified products were separated by electrophoresis on 1.2% agarose gel (SinaClon, Iran); after staining with ethidium bromide, they were visualized under UV gel documentation system; then they were photographed and compared together by visual inspection.

2.6. Statistical analysis

The results were entered and analyzed using the SPSSTM software, version 22.0 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft Corporation, USA) statistical software to obtain frequencies and comparison among clones. *Chi*-square test was applied to analyze intergroup significance. In addition, $P< 0.05$ was considered statistically significant and the confidence interval was 95%.

3. Results

3.1. Occurrence of A. baumannii

A total of 121 (16.1%) non-duplicate *A. baumannii* isolates were collected and identified in 750 clinical specimens from different wards of Imam Hossein and Imam Khomeini Hospital in Tehran, Iran. The isolates were obtained from 56 (46.3%) males and 65 (53.7%) females with the mean age of 39.5 years (ranged 9–79 years). The presence of $bla_{\text{OXA-51-like}}$ gene was confirmed in all isolates by PCR. The majority of the isolates were collected from intensive care unit (49.6%) and surgery (14.0%), respectively. The frequencies of collected isolates in terms of the hospital ward and clinical samples are mentioned in Table 2.

3.2. Antimicrobial susceptibility rate

The results of susceptibility testing showed that the colistin with 100% sensitivity rate had the highest effect, while ceftriaxone with 16.5% sensitivity rate had the least effect on *A. baumannii* isolates. The tigecycline with 77.6% sensitivity rate was the second most effective antibiotic after colistin. Additionally, more than 60% of the isolates were resistant against pipracillin/tazobactam, tetracycline, amikacin, cefepime, tobramycin, and ciprofloxacin. Among 121 *A. baumannii* strains, 96 (79.3%) and 99 (81.8%) isolates were resistant to imipenem and meropenem, respectively. Ninety-six (79.3%) isolates were simultaneously resistant to imipenem and meropenem. In total, 99 (81.8%) of *A. baumannii* strains were carbapenem resistant. The results of the susceptibility rate of all tested antibiotics are displayed in Table 3. The results revealed that all *A. baumannii* strains collected from the trachea, wound, and urine samples were resistant to all antibiotics except for colistin, but the strains isolated from the pleural fluid were susceptible to the majority of antibiotics (Figure 1).

3.3. Multidrug resistance patterns

MDR isolates were determined based on their resistance to at least one antibiotic in three different antimicrobial categories. A total of 109 isolates (90.0%) were MDR with 10 different resistotypes (Table 4). The R4 with the rate of 24.8% was the most prevalent resistotype.

3.4. Molecular characterization of oxacillinase genes

The obtained results from multiplex PCR revealed that 77 (77.7%) isolates of 99 carbapenem resistant *A. baumannii* had positive PCR band for at least one of $bla_{\text{OXA-like}}$ genes. In total, 75 (75.7%) of carbapenem resistant isolates were positive for $bla_{\text{OXA-23-like}}$, and 14 (14.1%) for $bla_{\text{OXA-24-like}}$ gene. Meanwhile, the $bla_{\text{OXA-51-like}}$ gene was detected in all tested strains. The co-existence of $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ genes were found in 12 (12.1%) isolates. Finally, the $bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-143-like}}$ genes were not detected in any isolates.

Table 2. Frequency of collected *Acinetobacter baumannii* strains in terms of the ward and samples (n=121).

Items	n	%
Clinical samples		
Wound	17	14.0%
Blood	10	8.3%
Trachea	46	38.0%
Urine	16	13.2%
Cerebrospinal fluid	12	9.9%
Pleural fluid	5	4.1%
Catheter	15	12.4%
Wards		
Pediatric	13	10.7%
Infectious diseases	9	7.4%
General	4	3.3%
Intensive care unit	60	49.6%
Surgery	17	14.0%
Urology	10	8.3%
Neurology	8	6.6%

Table 3. Resistance rate of *Acinetobacter baumannii* strains to the tested antibiotics [n (%)].

Antibiotics	Resistant	Susceptible
Meropenem	99 (81.8)	22 (18.2)
Tobramycin	78 (64.5)	43 (35.5)
Cefepime	80 (66.1)	41 (33.9)
Ceftriaxone	101 (83.5)	20 (16.5)
Amikacin	85 (70.2)	36 (29.8)
Ciprofloxacin	96 (79.3)	25 (20.7)
Pipracillin/tazobactam	101 (83.5)	20 (26.5)
Imipenem	96 (79.3)	25 (20.7)
Tetracycline	75 (61.9)	46 (38.1)
Colistin	0 (0.0)	121 (100.0)
Tigecycline	27 (12.4)	94 (77.6)

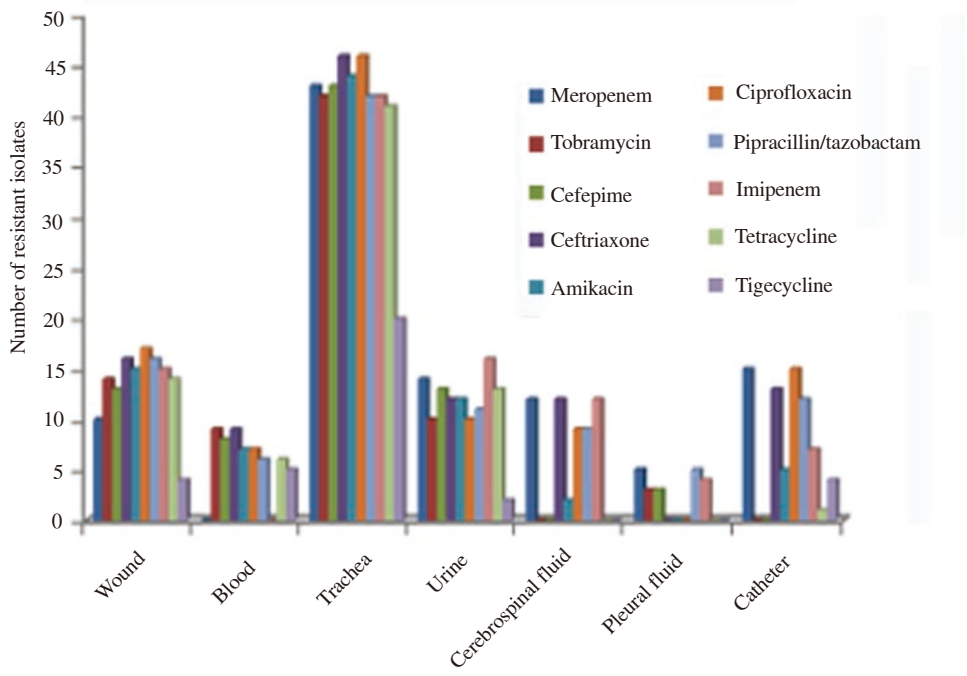

Figure 1. Antimcrobial resistance of *Acinetobacter baumannii* in clinical specimens.

Table 4. Resistotypes of *Acinetobacter baumannii* strains based on tested antibiotics categories.

Resistotypes	C1	C2		C3		C4		C5	C6	C7	C8	*n*	%
	PTZ	CRO	FEP	IMI	MEM	AN	TM	CO	CIP	TET	TGC		
R1	R	R	R	R	R	R	R	-	R	R	-	20	18.3
R2	R	R	R	R	R	R	R	-	R	R	R	15	13.8
R3	R	R	R	R	R	R	R	-	R	-	R	5	4.6
R4	R	R	-	R	R	R	-	-	R	-	-	27	24.8
R5	-	R	R	R	R	R	R	-	-	R	R	4	3.7
R6	R	R	R	R	R	-	R	-	R	R	-	25	22.9
R7	R	R	R	-	-	R	R	-	R	R	R	9	8.3
R8	-	R	R	-	R	R	-	-	R	R	R	1	0.9
R9	-	R	-	-	R	R	-	-	R	-	-	2	1.8
R10	-	-	R	-	-	R	-	-	-	R	R	1	0.9

C1: penicillins; C2: extended-spectrum cephalosporins; C3: carbapenems; C4: aminoglycoside; C5: polymyxins; C6: fluoroquinolones; C7: tetracyclines; C8: Glycylcycline; PTZ: pipracillin/tazobactam; CRO: ceftriaxone; FEP: cefepime; IMI: imipenem; MEM: meropenem; AN: amikacin; TM: tobramycin; CO: colistin; CIP: ciprofloxacin; TET: tetracycline; TGC: tigecycline.

Table 5. Resistance rate of bla_{OXA}-like producer and non-producer *Acinetobacter baumannii* isolates [*n* (%)].

Antibiotics	bla_{OXA}-producer *n* (%)		bla_{OXA}-nonproducer *n* (%)		χ^2	*P*-value
	Resistant	Susceptible	Resistant	Susceptible		
Meropenem	69 (89.6)	8 (10.4)	30 (34.1)	14 (65.9)	40.657	< 0.001
Tobramycin	54 (70.1)	24 (54.5)	23 (29.9)	20 (45.5)	2.969	0.064
Cefepime	50 (64.9)	27 (35.1)	30 (68.2)	14 (31.8)	0.132	0.437
Ceftriaxone	61 (79.2)	16 (20.8)	40 (90.9)	4 (9.1)	2.773	0.076
Amikacin	50 (64.9)	27 (35.1)	35 (79.5)	9 (20.5)	2.860	0.067
Ciprofloxacin	57 (74.0)	12 (26.0)	39 (88.6)	5 (11.4)	3.646	0.044
Pipracillin/tazobactam	70 (90.9)	7 (9.1)	31 (70.5)	13 (29.5)	8.491	0.004
Imipenem	66 (85.7)	11 (14.3)	30 (68.2)	14 (31.8)	5.251	0.021
Tetracycline	48 (62.3)	29 (37.7)	27 (61.4)	17 (38.6)	0.011	0.534
Colistin	0 (0.0)	77 (100.0)	0 (0.0)	44 (100.0)	-	-
Tigecycline	16 (20.8)	61 (79.2)	11 (25.0)	33 (75.0)	0.288	0.375

3.5. Resistance patterns of blaOXA-like producer A. baumannii isolates

Antibiogram results of $bla_{\text{OXA-like}}$ producer and non-producer *A. baumannii* isolates are shown in Table 3. Based on the results, there was a significant difference in the resistance to imipenem, meropenem, ciprofloxacin, and pipracillin/tazobactam antibiotics between $bla_{\text{OXA-like}}$ producer and non-producer *A. baumannii* isolates (Table 5).

3.6. REP-PCR molecular typing

The molecular typing of 99 carbapenem resistant *A. baumannii* isolates by REP-PCR revealed 9 dissimilar clones (A-I). The five main clones A, B, C, D, and E were detected in 25 (25.2%), 36 (36.4%), 10 (10.1%), 8 (8.0%), and 6 (6.1%) of isolates, respectively. The frequency of other clusters were as follows: 4 isolates (4.0%) for clone F, 1 isolate (1.0%) for clone G, 4 isolates (4.0%) for clone H, and 5 isolates (5.1%) for clone I.

4. Discussion

Nowadays, *A. baumannii* has become a global challenge in terms of therapeutic choices due to the resistance to various antibiotics. Therefore, careful screening of the resistance pattern of this bacterium is very important in health care centers and hospitals. In this study, as expected, similar to previous studies from Iran and different countries, the polymyxins and glycylcycline antibiotic groups were the most effective anti-bacterial drugs against *A. baumannii* isolates[19-21].

This can be due to the low prescription of these drug categories and their reserve as the last line of infection therapy in our region. Additionally, the prevalence of resistance rate of polymyxins and glycylcycline among *A. baumannii* isolates was similar to the global report that mentioned the pooled prevalence of 0.0-3.7 and 2.3-25.8 for colistin and tigecycline, respectively[22]. Also, similar to studies reported by Shoja *et al.*[19] and Sarikhani *et al.*[23] from Iran, a high resistance rate above 80% was seen against third-generation cephalosporins in our study.

Resistance to carbapenems in *A. baumannii* strains is an increasingly remarkable phenomenon leading to limitation of treatment choices. Several reports from different regions of Iran indicated a trend toward increasing resistance to carbapenems in clinical isolates of *A. baumannii*[24]. In the current study, the results of antibiogram revealed that 18.2% of *A. baumannii* isolates were susceptible to carbapenems which was in line with other studies from different geographic regions of the world that reported an 8%–26% susceptibility rate for imipenem among *A. baumannii isolates*[25].

The high resistance rate of *A. baumannii* isolates against meropenem (81.8%) and imipenem (79.3%) in current study was consistent with previous studies from Iran[18,22]. In a recent study by Mobasseri *et al.*[26] from Iran a resistance rate of 96% were reported against both imipenem and meropenem antibiotics in clinical isolates of *A. baumannii*.

These findings reflect the fact that the prescription of aforementioned antibiotics for the treatment of infections caused by the *A. baumannii* should be done with caution and based on the results of *in vitro* antimicrobial susceptibility testing. Another finding of the current study was the high percentage (90%) of multi-drug resistance among *A. baumannii* isolates, which confirmed the results of other studies from around the world[27,28].

The carbapenem resistance in *A. baumannii* is caused by various mechanisms, including class D beta-lactamase enzymes. After discovering the OXA-23 the first class D beta-lactamase in a strain of *A. baumannii*, other oxacillinases with carbapenemase activity have been characterized[29]. The present study demonstrated the presence of intrinsic OXA-51 oxacillinase in all isolates. Despite its poor anti-carbapenem activity, when this enzyme is overproduced, it is able to increase the minimum inhibitory concentration of carbapenems[30].

The OXA-23, OXA-24, OXA-58, OXA-143, and OXA-72 are most abundant acquired oxacillinases that have been observed in *A. baumannii* isolates[30]. In this study, the multiplex PCR method was performed to investigate the frequency of OXA-23, OXA-24, OXA-58, and OXA-143 oxacillinases in carbapenem resistant *A. baumannii* isolates. Not surprisingly, the evidence from this research showed the highest frequency of OXA-23 oxacillinase (75.7%) in studied isolates that was in good agreement with earlier studies from Iran[19,23].

However, in this study, 14.1% of *A. baumannii* isolates were positive for OXA-24 oxacillinase which was in contrast to the findings of Shoja *et al.*[19] and Karmostaji *et al.*[31] that reported lower occurrence rate for this oxacillinase. Furthermore, in another study from Brazil, this oxacillinase was not found in any carbapenem resistant *A. baumannii* isolates that was contrary to present study[21]. Unlike a research carried out by Sarikhani *et al.*[23] in this area in Iran, we did not detect the OXA-58 and OXA-143 oxacillinases in *A. baumannii* isolates. They reported an occurrence rate of 55.6% and 14.4% for OXA-58 and OXA-143, respectively.

However, our results were consistent with that of Shoja *et al.*[19] and Mohajeri *et al.*[32] that did not detect the OXA-58 in their isolates. Meanwhile, consistent with previous reports from Algeria[33] that mentioned the co-existence of oxacillinases in *A. baumannii* isolates; this research found that 12.1% of *A. baumannii* isolates have co-existence of two OXA-23 and OXA-24 oxacillinases.

It should be noted that in this study, 22 isolates from all 99 carbapenem resistant *A. baumannii* lacked the acquired oxacillinases. The mechanisms of carbapenem resistance in these isolates may be raised from porin loss, specific efflux pump, or presence of metallo-β-lactamases such as the NDM, IMP and VIM types[34,35].

5. Conclusion

The main aim of this study was to achieve a baseline background to determine the resistant pattern of oxacillinase producing *A. baumannii* in clinical samples. Result from this study affords valuable knowledge to perform further large prospective study to identify the other mechanisms involved in carbapenem resistant *A. baumannii* isolates.

The OXA-23 was the main oxacillinase that contributed to carbapenem resistance in our study. Observing the high frequency of multiple drug resistant *A. baumannii* isolates in this study, highlighted the need for continuous and regularly monitoring of antibiotic resistance patterns of these bacteria in our region to control the spread of MDR pathogens in the healthcare facilities.

Conflict of interest statement

The authors report no conflict of interest.

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Authors' contribution

M.A.M., M.D., A.J., B.G.S., M.K.Z.: Conceived and designed the experiments; performed the experiments; contributed reagents, materials, analysis tools or data and interpreted. M.D., A.J., B.G.S. and S.K.: Analyzed and interpreted the data; contributed reagents, materials and wrote the paper.

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