ORIGINAL ARTICLE

Evaluating the frequency of carbapenem and aminoglycoside resistance genes among clinical isolates of *Acinetobacter baumannii* from Ahvaz, south-west Iran

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Abstract

Acinetobacter baumannii is one of the most important opportunistic challenging pathogens as a result of its ability to acquire resistance to broad range of antibiotics and cause a variety of severe nosocomial infections. We investigated the frequency of the aminoglycoside-modifying enzymes (AMEs) and oxacillinase genes among clinical isolates of *A. baumannii* collected from hospitalized patients in Imam Khomeini Hospital, Ahvaz city, Iran. This prospective cross-sectional study was performed on 80 clinical isolates of *A. baumannii* collected from patients referred to Imam Khomeini Hospital in Ahvaz, Iran. Initial identification of isolates as *A. baumannii* was performed using conventional bacteriologic tests, and final confirmation was carried out by PCR of bla_{OXA-51}-like gene and multiplex PCR of *gyrB* locus. MICs of different classes of antibiotics against these strains was measured by using VITEK 2 system. After extraction of genomic DNA, two groups of multidrug-resistant *A. baumannii* genes including AME (*aadA1*, *aadB*, *aphA6* and *aacC1*) and oxacillinases (bla_{OXA-23}-like, bla_{OXA-24}-like, bla_{OXA-51}-like, bla_{OXA-58}-like and $bla_{OXA-143}$-like) were detected. According to antibiotic susceptibility testing, among 80 *A. baumannii* strains, 75 isolates (91.25%) were multidrug resistant. The results showed that colistin and tigecycline, with respective sensitivity rates of 97.5% (78/80) and 56.25% (45/80), had the highest effects. The presence of bla_{OXA-51}-like and *gyrB* genes was confirmed in all strains. Furthermore, bla_{OXA-23}-like and bla_{OXA-24}-like genes were found in 68.75% (55/80) and 20% (16/80) of isolates respectively, while no isolate harbored the $bla_{OXA-143}$-like gene. The frequency of genes encoding the AMEs including *aadA1*, *aacC1*, *aphA6* and *aadB* were 11.25% (9/80), 16.25% (13/80), 22.5% (18/80) and 30% (24/80) respectively. Our findings indicate that the presence of the *aadB* and *aphA6* is correlated with high resistance against amikacin and gentamicin. We found a very high resistance rate against most of the antimicrobial agents usually prescribed for severe infections caused by *A. baumannii.* Therefore, because of rapid emergence of resistance even for colistin or tigecycline, monotherapy should be avoided. These results show the importance of providing antibiotics correctly in intensive care units and following antibiotic stewardship protocols as the only effective strategies to attempt to control antibiotic resistance in healthcare settings.

Keywords: *Acinetobacter baumannii*, aminoglycoside resistance genes, carbapenem-resistant *A. baumannii* (CRAB), multidrug resistance (MDR), oxacillinase genes

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Introduction

Acinetobacter baumannii is an opportunistic pathogen that can survive for long periods on both dry and moist surfaces. *A. baumannii* is prevalent in healthcare facilities, colonizes different surfaces and survives on the hair or skin of patients

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and hospital staff as a commensal bacterium. The ability of *A. baumannii* to survive in hospital environments for a long time and its ability to gain many virulence factors have led it to emerge as an important nosocomial pathogen [1,2].

Multidrug resistance (MDR) is defined as resistance to three or more representatives of the following classes of antibiotics: carbapenems, aminoglycosides, third-generation cephalosporins and fluoroquinolones. The rapid spread of MDR isolates of *A. baumannii* causing nosocomial infections is of great global concern [3]. Carbapenems are members of the β-lactam class of antibiotics, which are used as a drug of choice for treatment of infections due to MDR *A. baumannii* isolates, but recently the rise of resistance to this class of antibiotics has led to limited efficiency of these drugs [4].

Carbapenem-resistant *A. baumannii* is a major cause of nosocomial infections worldwide, leading to high mortality and morbidity and consequently increased medical costs. Resistance to carbapenem in *A. baumannii* strains is mediated by acquisition of a class B or class D β-lactamase genes such as metallo-β-lactamases and oxacillinase genes respectively. OXA-type carbapenamases have been divided into eight subgroups, with six identified in *A. baumannii*: OXA-23, OXA-40, OXA-51, OXA-58, OXA-143 and OXA-235 [5,6].

Overuse of carbapenems is an important factor for the development of colonization with carbapenem-resistant *A. baumannii* strains. The spread of carbapenem-resistant MDR *A. baumannii* has led to the use of polymyxins, particularly polymyxin E (colistin). Colistin, a polypeptide antibiotic, targets the bacterial outer membrane. Various mechanisms, including plasmid-mediated *mcr-1* gene, mutations in the *pmrA*, *pmrB*, *lpxA*, *lpxC* and *lpxD* genes and the presence of insertion sequence IS*Aba11* in either *lpxC* or *lpxA*, are involved in the emergence of resistance to this antibiotic [7].

Aminoglycoside antibiotics have long been used for the therapy of infection in patients and are still a drug of choice for the treatment of diseases caused by MDR strains. Many researches show that *A. baumannii* can acquire drug resistance against these antibiotics [8]. Drug efflux pumps, methylases, 16S ribosomal RNA and AMEs play important roles in resistance to aminoglycoside. AMEs target different sites of these antibiotics [9,10]. The type of reaction assigns the nomination of AMEs, such as nucleotidyltransferases (ANT), phosphotransferase (APH) and acetyltransferase (AAC). The genes encoding AMEs may be located on class 1 integrons, transposons and plasmids in MDR *A. baumannii* [11].

The aims of this study were to investigate the frequency of the AME and oxacillinase genes among clinical isolates of *A. baumannii* recovered from hospitalized patients in Imam Khomeini Hospital in Ahvaz, Iran.

Materials and methods

Study design, data and specimens collection

This prospective cross-sectional study was performed between January 2018 and November 2019. Written informed consent was obtained from all patients (study approval IR.MUBAM.REC.1398.072). The present research was carried out by using 80 strains of *A. baumannii* isolated from patients referred to Imam Khomeini Hospital in Ahvaz, south-west Iran. These strains were isolated from blood, urine, catheter, cerebrospinal fluid and trachea aspiration samples.

All samples were transported to the microbiology laboratory within 4 hours of collection and were processed immediately. In the following, the isolates were identified on the basis of standard bacteriologic tests, including Gram staining, oxidase, growth on MacConkey and blood agars, sugar fermentation on triple-sugar iron agar, methyl red, Voges-Proskauer, motility, Simmons citrate agar and urease [12]. Finally, confirmation was conducted by PCR of $bla_{\text{OXA-51}}$-like gene and multiplex of *gyrB* [13]. The *A. baumannii* ATCC19606 strain was used as positive control.

Antimicrobial susceptibility testing

MICs of the following antibiotics was established via the VITEK 2 system (bioMérieux, Marcy l'Etoile, France). The antibiotics were as follows: ampicillin/sulbactam, piperacillin, cefepime, ceftazidime, cefotaxime, amikacin, meropenem, imipenem, ciprofloxacin, levofloxacin, minocycline, gentamicin, tobramycin, tetracycline, colistin, tigecycline and trimethoprim/sulfamethoxazole. The MICs of all the antibiotics were interpreted by the system according to Clinical and Laboratory Standards Institute guidelines [14].

A MIC $\geq$4 μg/mL for colistin was considered as the breakpoint of resistance as well as a MIC $\geq$8 μg/mL for imipenem and meropenem. *Acinetobacter* isolates are defined as MDR strains resistant to at least three classes of antimicrobial agents, including aminoglycosides, penicillins and cephalosporins and fluoroquinolones.

Molecular detection of oxacillinase and AME genes

Genomic DNA used as a template for PCR assays was extracted from isolates by boiling [15]. We investigated two groups of MDR *A. baumannii* genes: AME (*aadA1*, *aadB*, *aphA6* and *aacC1*) and oxacillinases ($bla_{\text{OXA-23}}$-like, $bla_{\text{OXA-24}}$-like, $bla_{\text{OXA-51}}$-like, $bla_{\text{OXA-58}}$-like and $bla_{\text{OXA-143}}$-like). The reactions were performed in 25 μL volume. The amplification mixture consisted of 200 μM deoxynucleotide triphosphates, 2.5 μL tenfold concentrate PCR buffer, 2.5 mM $MgCl_2$, 0.1 μM of each

TABLE 1. Primers used for gene amplification

Target gene	Sequence (5′–3′)	Amplicon size (bp)	Reference
bla_{OXA-51}	F-TAATGCTTTGATCGGCCTTG R-TGGATTGCACTTCATCTTGG	353	[16]
bla_{OXA-23}	F-GATCGGATTGGAGAACCAGA R-ATTTCTGACCGCATTTCCAT	501	[16]
bla_{OXA-24}	F-GGTTAGTTGGCCCCCTTAAA R-AGTTGAGCGAAAAGGGGATT	246	[16]
bla_{OXA-58}	F-AAGTATTGGGGCTTGTGCTG R-CCCCTCTGCGCTCTACATAC	599	[16]
$bla_{OXA-143}$	F-TGGCACTTTCAGCAGTTCCT R-TAATCTTGAGGGGGCCAACC	149	[17]
aphA6	F-ATGGAATTGCCCAATATTATTC R-TCAATTCAATTCATCAAGTTTTA	797	[18]
aacC1	F-ATGGGCATCATTCGCACATGTAGG R-TTAGGTGGCGGTACTTGGGTC	465	[18]
aadB	F-ATGGACACAACGCAGGTCGC R-TTAGGCCGCATATCGCGACC	534	[18]
aadA1	F-ATGAGGGAAGCGGTGATCG R-TTATTTGCCGACTACCTTGGTG	792	[18]

primer, 1.5 U of Taq DNA polymerase (CinnaGen, Tehran, Iran); and finally 2 μL DNA was added to this mixture. Primer sequences used for the detection of these genes are presented in Table 1. The amplification conditions for oxacillinase genes were as follows: initial denaturation for 5 minutes at 94°C, 35 cycles of 94°C for 25 seconds, 52°C for 40 seconds and 72°C for 50 seconds. The final extension was performed at 72°C for 3 minutes. PCR conditions for AME genes included 35 cycles of amplification under the following conditions: denaturation at 95°C for 3 minutes; annealing at 53–55°C for 30 seconds; and cycling followed by 45 seconds' extension at 72°C, with a final extension at 72°C for 5 minutes. PCR products were resolved on 2.0% agarose gels, stained with safe stain and photographed by UV illumination.

Data analysis

The results were analysed by SPSS 22 (IBM, Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft, Redmond, WA, USA) software. We considered $p < 0.05$ to be statistically significant, and 95% confidence intervals were used. The results are presented as descriptive statistics in terms of relative frequency.

Results

Bacterial isolates

A total of 80 *A. baumannii* strains were collected from the different clinical samples, including tracheal aspiration in 32 (36.25%), blood in 20 (25%), catheter in 16 (20%), urine in eight (10%) and cerebrospinal fluid in four (5%) isolates. Forty-six patients (57.5%) were male and 34 (42.5%) female; mean ± standard deviation age was 30 ± 1 years, with a range of 2 to 65 years. The frequency of collected *A. baumannii* isolates from different wards of the hospital was intensive care unit (ICU) in 31 (38.75%), urology in 20 (25%), general in ten (12.5%), surgery in six (7.5%), infectious diseases in seven (8.75%) and paediatric in six (7.5%) respectively (Table 2).

Antibiotic susceptibility

According to antibiotic susceptibility testing, among 80 *A. baumannii* strains screened, 75 isolates (91.25%) were MDR. The effect of the antimicrobial agents assessed in the present study against *A. baumannii* strains is listed in Table 3. The results of showed that colistin and tigecycline, with respective sensitivity rates of 78 (97.5%) and 45 (56.25%), had the highest effect. Seventy-two isolates (90%) were resistant to gentamicin and amikacin simultaneously. More than 70% of the isolates were resistant to ciprofloxacin, amikacin, piperacillin, tetracycline, ampicillin/sulbactam, trimethoprim/sulfamethoxazole and gentamicin. Resistance to cephalosporins was higher than 90%. Also, 73 isolates (91.25%) and 64 isolates (80%) were resistant to imipenem and meropenem respectively. Sixty-three isolates (78.75%) were resistant to meropenem and imipenem simultaneously. The lowest level of MIC for imipenem was 0.5 μg/mL and the highest level was 512 μg/mL. Most of the meropenem-resistant isolates (29.68%) had meropenem MIC 16 μg/mL. Among strains sensitive to colistin, 13 isolates (16.25%) and two isolates (2.5%) had MIC 1 μg/mL and 2 μg/mL respectively. Two strains (Ab4 and Ab41) were resistant to colistin with MIC 32 and 16 μg/mL.

Molecular characterization of oxacillinase

The presence of bla_{OXA-51}-like and *gyrB* genes was confirmed in all strains. Also, bla_{OXA-23}-like and bla_{OXA-24}-like genes were found in 68.75% (55/80) and 20% (16/80) respectively, while no isolate harbored the $bla_{OXA-143}$-like gene. Also, the combination of bla_{OXA-24}-like/bla_{OXA-58}-like/bla_{OXA-23}-like and bla_{OXA-24}-like/bla_{OXA-23}-like genes were seen in three (Ab8, Ab18 and

TABLE 2. Characteristics of *Acinetobacter baumannii* strains

Strain identification	Ward	Sample	MIC (μg/mL)			OXA type	Gene	Resistance to AMK/GEN
			MER	IMI	COL			
Ab1	General	TA	16	32	0.5	24	*A1*	+/+
Ab2	ICU	Blood	32	128	0.5	23	—	+/−
Ab3	Urology	TA	64	16	0.5	23	*B, A6*	+/+
Ab4	ICU	Catheter	16	512	32	23	*A6*	+/+
Ab5	Surgery	CSF	128	32	0.25	24	*A1*	−/−
Ab6	Urology	TA	1	1	0.25	23, 24	*B*	+/+
Ab7	ICU	Blood	32	16	0.125	23	*C1*	+/+
Ab8	ICU	Catheter	16	128	0.5	23, 24, 58	*A6*	+/+
Ab9	General	TA	32	64	1	23	*B, C1, A6*	+/+
Ab10	Urology	Catheter	64	128	1	23	*B*	+/+
Ab11	ID	TA	1	1	0.5	23	*C1*	+/+
Ab12	ICU	Blood	16	128	0.25	23	—	+/+
Ab13	Urology	Urine	32	32	0.5	24	*B*	+/+
Ab14	Paediatric	TA	16	64	0.125	23	*A6*	+/+
Ab15	ICU	Blood	32	256	0.5	23	*C1*	+/+
Ab16	General	TA	128	128	1	23	*A1*	+/+
Ab17	Paediatric	Catheter	1	0.5	0.5	23	*B*	+/+
Ab18	Surgery	CSF	16	128	1	23, 24, 58	*C1*	+/+
Ab19	Urology	TA	64	16	1	23	*A6*	+/+
Ab20	ICU	Blood	0.5	256	0.5	23	—	+/−
Ab21	ICU	TA	32	64	0.5	23	*B, A6*	+/+
Ab22	ID	TA	16	128	1	24	*A1*	+/+
Ab23	Urology	Blood	64	32	0.25	23	*B*	−/−
Ab24	General	Urine	2	256	0.25	24	*A6*	−/+
Ab25	ICU	Blood	32	128	0.5	23	—	+/+
Ab26	Paediatric	TA	1	2	2	23, 24	*B*	+/+
Ab27	Paediatric	Urine	64	64	1	23	*B*	+/+
Ab28	ICU	TA	16	32	0.5	24	*B, A6*	+/+
Ab29	ICU	TA	64	32	0.5	23	*B*	+/+
Ab30	Urology	Blood	0.5	256	0.25	23, 58	*A1*	−/+
Ab31	General	TA	64	128	0.125	23	*B*	+/+
Ab32	ICU	Catheter	128	16	0.25	23	*A6*	+/+
Ab33	Urology	Urine	16	128	0.25	24	*B*	+/+
Ab34	Urology	Blood	2	64	1	23	*A6*	+/+
Ab35	Surgery	TA	128	32	0.5	23	—	+/+
Ab36	ICU	Blood	32	128	2	23, 24	*B, A6*	+/+
Ab37	Paediatric	Catheter	128	16	0.25	23	*C1*	+/+
Ab38	ID	TA	64	128	0.5	24	*B*	+/+
Ab39	ICU	Catheter	32	256	1	23	*A6*	+/+
Ab40	Urology	TA	0.5	32	0.25	23	*B*	+/+
Ab41	ICU	Blood	128	256	16	23	*A6*	−/+
Ab42	ICU	TA	16	32	0.25	23	*B*	+/+
Ab43	General	Catheter	64	64	0.25	24	*B*	+/+
Ab44	Urology	Blood	32	32	0.5	23	*A1*	−/+
Ab45	ICU	TA	64	256	0.25	23	*A6*	+/+
Ab46	ID	Catheter	0.5	1	0.5	23	*C1*	+/−
Ab47	ICU	TA	16	128	0.125	24	*B*	+/+
Ab48	Urology	TA	128	256	0.25	23	*C1*	+/+
Ab49	Surgery	Blood	64	32	0.25	23	*B*	+/+
Ab50	ICU	CSF	16	16	0.5	23	*B, A6*	+/+
Ab51	ID	Blood	128	32	0.5	23	*A6*	+/+
Ab52	ICU	CSF	256	64	1	23, 24	*C1*	+/−
Ab53	General	TA	16	128	0.25	24	*A6*	+/+
Ab54	Urology	Catheter	16	32	0.5	23	—	−/+
Ab55	ICU	Urine	128	32	0.5	23	*A1*	+/+
Ab56	Paediatric	TA	256	16	0.25	23	*B*	−/+
Ab57	ICU	Blood	0.5	128	0.25	23, 58	*A6*	−/+
Ab58	ICU	Catheter	32	64	0.25	24	*B*	+/+
Ab59	Urology	TA	32	128	0.5	23	*B*	+/+
Ab60	ID	TA	128	256	0.25	23, 24, 58	*B*	+/+
Ab61	ICU	Catheter	1	0.5	1	23	*B, C1, A6*	+/+
Ab62	Paediatric	TA	16	256	0.5	24	*C1*	+/+
Ab63	ICU	TA	512	32	0.5	23	*A6*	+/+
Ab64	Urology	Catheter	2	32	0.25	23	*A1*	+/+
Ab65	Urology	Blood	32	16	0.5	23	*B, A6*	+/+
Ab66	General	TA	256	64	0.5	24	*B*	+/+
Ab67	Surgery	Catheter	16	512	0.5	23	*C1*	−/−
Ab68	ICU	TA	128	64	1	23	*A6*	+/+
Ab69	Urology	Urine	0.5	128	1	23	—	+/−
Ab70	ICU	Catheter	16	64	0.5	23	*A1*	+/+
Ab71	ID	Blood	32	32	0.25	24	*C1*	−/+
Ab72	Urology	Blood	128	128	0.25	23	*A6*	+/+
Ab73	General	Urine	16	16	0.5	23	*B*	+/+
Ab74	ICU	TA	1	32	0.25	23	*B*	+/+
Ab75	Urology	Blood	32	0.5	0.5	23	*C1*	−/+
Ab76	Surgery	Catheter	32	256	0.125	23	*A6*	+/+
Ab77	ICU	TA	128	128	0.25	24	—	+/+
Ab78	Urology	Blood	16	32	0.25	23	*B*	−/−
Ab79	General	Urine	128	64	0.5	23	*A6*	+/+
Ab80	ICU	TA	0.5	32	0.5	23	*C1*	+/+

A1, *aadA1*; A6, *aphA6*; AMK, amikacin; B, *aadB*; C1, *aacC1*; COL, colistin; GEN, gentamicin; ID, infectious disease; M, medium; MDR, multidrug resistant; MER, meropenem; N, non–biofilm forming; PF, pleural fluid; S, strong; TA, tracheal aspirate; TGC, tigecycline; W, weak; XDR, extensively drug resistant.

TABLE 3. Antibiotic susceptibility pattern of *Acinetobacter baumannii* isolates

Susceptibility pattern	COL	TGC	IMI	MER	CTZ	DFP	CTX	CIP	TET	PIP	SXT	AMP/S	AMK	GEN
Resistant	71 (88.75)	67 (83.75)	63 (78.75)	76 (95.0)	77 (96.25)	55 (72.5)	74 (92.5)	78 (97.5)	80 (100)	75 (93.75)	64 (80.0)	73 (91.25)	35 (43.75)	2 (2.5)
Intermediate	0	0	0	2 (2.5)	0	0	2 (2.5)	0	0	0	0	0	0	0
Susceptible	9 (11.25)	13 (16.25)	17 (21.25)	2 (2.5)	3 (3.75)	25 (27.5)	4 (5.0)	2 (2.5)	0	5 (6.25)	16 (20)	7 (8.75)	45 (56.25)	78 (97.5)

Data are presented as *n* (%). AMK, amikacin; AMP/S, ampicillin/sulbactam; CFP, cefepime; CIP, ciprofloxacin; COL, colistin; CTX, cefotaxime; CTZ, ceftazidime; GEN, gentamicin; GEN, gentamicin; IMI, imipenem; LEV, levofloxacin; MEM, meropenem; MIN, minocycline; PIP, piperacillin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TGC, tigecycline; TGC, tigecycline; TOB, tobramycin.

Ab60) and four (Ab6, Ab26, AB36 and Ab52) of the isolates respectively. The coexistence of the bla_{OXA-58}-like and bla_{OXA-23}-like genes occurred in two isolates (Ab30 and Ab57). In isolates with the bla_{OXA-24}-like and bla_{OXA-23}-like genes, 68.75% (11/80) and 47.27% (26/80) of isolates had imipenem MICs of 64 µg/mL or more respectively. In isolates with bla_{OXA-24}-like and bla_{OXA-23}-like genes, 31.25% (5/16) and 40% (22/55) had meropenem MICs of 64 µg/mL or more respectively (Table 4).

AME genes

All strains were screened for the presence of genes encoding the AMEs. Screening of these genes by PCR showed that frequency of *aadA1*, *aacC1*, *aphA6* and *aadB* were 11.25% (9/80), 16.25% (13/80), 22.5% (18/80) and 30% (24/80) respectively. Two strains (7.5%) had *aadB*, *aacC1* and *aphA6* genes, and six strains had *aadB* and *aphA6* genes. Also, eight strains did not have any AME genes. All strains with two or three AME genes were 100% resistant to gentamicin and amikacin. In addition, resistance to these antibiotics in strains with one AME gene was more than 60%. Analysis of coexistence of AME and genes encoding OXA carbapenemases indicated that more than 50% of strains with AME genes had bla_{OXA-23}-like genes (Table 4). In the four isolates that were resistant to gentamicin and amikacin, no AME genes were detected.

Discussion

Nosocomial infections caused by MDR *A. baumannii* are a major problem worldwide, particularly in ICUs [19]. Our results shed light on the pressing issue of antimicrobial resistance among MDR *A. baumannii* isolates in Ahvaz, south-west Iran. In the present study, more than 38% of *A. baumannii* strains were isolated from samples obtained from patients in the ICU. As has been demonstrated, ICUs are high-risk areas for nosocomial infections because of the severity of underlying diseases, the use of invasive procedures and the duration of stay [20].

One of the main concerns in the treatment of *Acinetobacter* infections is the emergence of MDR and pandrug-resistant *Acinetobacter* isolates. Some outbreaks related to MDR *Acinetobacter* spp. have been recently described in several countries [21–23]. In the current study, we observed a high prevalence of the MDR phenotype (75/80, 91.25%) in *A. baumannii* isolates, a finding in agreement with other studies [24,25].

Carbapenems have become the favoured treatment for serious *Acinetobacter* infections in many centres and have retained better activity than other antimicrobial agents [26]. These antimicrobials have been utilized in Iran in most recent years, yet there is some evidence that increased and uncontrolled use of these agents favours the emergence of resistance to carbapenems [19]. Although we only tested susceptibility to imipenem and meropenem, we observed species that were highly resistant to carbapenems in this investigation, of which 91.2% were resistant to imipenem and 80% to meropenem. In a systematic review, the antimicrobial resistance rate of *A. baumannii* was assessed among 3409 samples collected from Iranian patients from 2001 to 2013 (51.1% imipenem, 64.3% meropenem) [27].

The investigation indicated a noteworthy increase in resistance rate against imipenem and meropenem, while resistance to aminoglycosides and lipopeptides did not have markedly change during these years. Resistance to carbapenems at the start of the research in 2001 was low (64.3% for meropenem and 51.1% for imipenem), but by the study's end in 2013, it reached 81.5% for meropenem and 76.5% for imipenem. Because of the high rate of *A. baumannii* resistance to studied antibiotics, they cannot be used as empirical treatment. Therefore, several studies have researched combination therapy of two or more agents for MDR *Acinetobacter* infections [28]. Recently tigecycline and colistin have developed as an alternative treatment choice for MDR *Acinetobacter* infections [29]. As shown in Table 3, colistin was the most active antimicrobial agent against most isolates of *A. baumannii*, with a susceptibility rate of 97.5%.

Because the frequency of resistance to colistin is low, it tends to be utilized as an easily accessible antibiotic for treatment of MDR *A. baumannii* strains susceptible to this agent [28,30]. In an investigation of 91 *A. baumannii* isolates from patients in tertiary-care ICUs of three university hospitals in

TABLE 4. Analysis of occurrence of aminoglycoside resistance genotypes, resistance patterns to amikacin (AMK), gentamicin (GEN) and genes encoding OXA carbapenemases among *Acinetobacter baumannii* strains

AME gene	*N* (%)	Resistance to AMK	Resistance to GEN	Resistance to AMK, GEN	bla_{OXA-23}-like	bla_{OXA-24}-like
aphA6	18 (22.5)	15 (83.3)	18 (100)	15 (83.3)	14 (77.7)	2 (11.1)
aadA1	9 (11.25)	6 (66.6)	8 (88.8)	6 (66.6)	5 (55.5)	3 (33.3)
aadB	24 (30)	21 (87.5)	22 (91.6)	21 (87.5)	14 (58.3)	7 (29.1)
aacC1	13 (16.25)	10 (76.9)	10 (76.9)	8 (61.5)	9 (69.2)	2 (15.3)
aadB, aphA6	6 (7.5)	6 (100)	6 (100)	6 (100)	4 (66.6)	1 (16.6)
aadB, aacC1, aphA6	2 (2.5)	2 (100)	2 (100)	2 (100)	2 (100.0)	0 (0.0)
Negative	8 (10)	7 (87.5)	5 (62.5)	4 (50)	7 (87.5)	1 (12.5)
Total		67%	71%	62%	55%	16%

Data are presented as *n* (%) unless otherwise indicated. AME, aminoglycoside-modifying enzyme; CSF, cerebrospinal fluid.

north, central and south Iran, the drug resistance pattern showed that 14.2%, 20% and 77% of the *A. baumannii* isolates were resistant to colistin, tigecycline and rifampicin respectively [24].

We demonstrated a rate of 2.5% for colistin-resistant *A. baumannii* isolates, which was lower than the resistance rate (5.6%) reported from north of Iran by Ezadi et al. [31]. In previous investigations in Pakistan [32] and Saudi Arabia [33], no colistin-resistant *A. baumannii* isolates were found in clinical samples. These diverse resistance rates can be explained by the patterns of administration, differences in the epidemiology of the regions and their use of antibiotics, as well as by dissimilarities in infection treatment regulatory policies.

The OXA carbapenemase genes are well distributed among the species of *Acinetobacter* spp. and have been well documented elsewhere [34,35]. These genes play a main role in the antibiotic resistance phenomenon among nosocomial bacteria and are reported on plasmids, integrons or transposons [36]. In the present study, similar to other studies conducted in Iran, OXA-23 was the most prevalent acquired OXA-type carbapenemase among carbapenem-resistant isolates, followed by OXA-24 [5,37,38]. This is not surprising because OXA-23 has been found around the world [39,40]. However, despite the abovementioned studies, combination bla_{OXA-24}-like/bla_{OXA-58}-like/bla_{OXA-23}-like and bla_{OXA-24}-like/bla_{OXA-23}-like genes were seen in our study. In a systematic review and meta-analysis on the prevalence of the carbapenem-resistant *A. baumannii* in Iran, the prevalence rates of OXA-23, OXA-24 and OXA-58 were reported as 73.7%, 21.9% and 6.2% respectively [41].

Like the study performed by Sarikhani et al. [42], we detected the bla_{OXA-58}-like gene in *A. baumannii* isolates. Sarikhani et al. reported an occurrence rate of 55.6% for this gene. However, our study results were not consistent with the results of previous research that did not detect the bla_{OXA-58}-like gene in isolates [43–45]. In the current study, the coexistence of the bla_{OXA-23}-like and bla_{OXA-58}-like genes occurred in two isolates (Ab30 and Ab57).

In agreement with our results, a study from Tehran noted the coexistence of bla_{OXA-23}-like/bla_{OXA-58}-like genes in two isolates (3%) [46]. In another study, the bla_{OXA-58}-like gene was identified in 30.95% of the studied isolates—remarkably higher than our study [47]. Also, in agreement with the current study regarding coexistence, Alavi-Moghaddam et al. [48], Tafreshi et al. [47] and Pournajaf et al. [45] reported coexistence rates of 12.1%, 3.57% and 17.4% respectively for bla_{OXA-23}-like and bla_{OXA-24}-like genes in their isolates.

Aminoglycosides are generally utilized for the treatment of *Acinetobacter* infection in Iran; however, at present, the increasing emergence of highly resistant strains is causing major concerns. Overall susceptibility rates to amikacin and gentamycin were 16.2% and 11.2% respectively. According to the molecular analysis of aminoglycoside-resistant strains, AMEs have been proposed as the principal mechanism related to aminoglycoside resistance [49].

The presence of highly frequent *aadA1* (11.25%), *aacC1* (16.25%) *aphA6* (22.5%) and *aadB* (30%) genes were demonstrated on clinical isolates of *Acinetobacter* by PCR. In a study from Tehran, screening of AME genes showed that frequency of *aadB*, *aphA6*, *aadA1* and *aacC1* genes was 72%, 65%, 37% and 21% respectively [50]. Gholami et al. [51] indicated that the *aac(6′)-Ib*, *aac(3)-I*, *aph(3′)-I* and *armA* genes are more prevalent than other genes; *aac(6′)-Id* and *rmtA* genes were found only at a very low incidence in the tested isolates. A study conducted by Xiao et al. [52] on *A. baumannii* isolates showed that the prevalence of *aac(3)-I, aac(6′)-Ib, ant(2″)-I and aph(3′)-I* genes were 10.7%, 17.9%, 14.3% and 17.9% respectively, and except for *armA* (17.9%), other types of 16S ribosomal RNA methylase genes were not detected in any of the isolates.

Conclusion

Our findings indicate that the presence of the *aadB* and *aphA6* genes was correlated with high resistance against amikacin and gentamicin. We also found a very high resistance rate against

most of the antimicrobial agents usually prescribed to treat infections. Monotherapy should be avoided because of the rapid emergence of resistance, even with colistin or tigecycline. Strict infection control strategies and antimicrobial resistance surveillance programmes are still lacking in Iran despite the alarming emergence of MDR *A. baumannii*, especially among colistin-resistant isolates. We hope these results change the attitude of physicians regarding utilizing antibiotics in ICUs; we encourage them to follow antibiotic stewardship guidelines as the only effective strategy to (somewhat) control antibiotic resistance in healthcare settings.

Conflict of interest

None declared.

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