



Carbapenem-resistant *Acinetobacter baumannii* isolates carrying *bla*_{OXA} genes with upstream *ISAb1*: First report of a novel OXA subclass from Iran

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ABSTRACT

Objectives: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) have emerged as a serious threat to public-health worldwide. This study aimed to determine the antimicrobial susceptibility of *A. baumannii* isolates in Iran and to investigate oxacillinase-encoding determinants and their association with insertion sequence *ISAb1* in CRAB isolates.

Methods: This study was performed on *A. baumannii* isolates recovered from patients with burn wound infections during 2013. All isolates were evaluated for antimicrobial susceptibility by the disk diffusion method. Minimum inhibitory concentrations (MICs) of five antibiotics (imipenem, meropenem, polymyxin B, colistin and tigecycline) were determined for all CRAB isolates. PCR was performed to determine the distribution of *bla*_{OXA} determinants and *ISAb1* insertion upstream of each corresponding gene in the CRAB isolates.

Results: A total of 65 *A. baumannii* isolates were recovered during the 1-year period, with CRAB accounting for 63 (96.9%) of isolates. Polymyxin B, colistin and tigecycline were the most effective agents against CRAB isolates, with susceptibility rates of 100%, 87.3% and 65.1%, respectively. The proportion of CRAB isolates carrying oxacillinase determinants was as follow: *bla*_{OXA-51-like}, 100%; *bla*_{OXA-23-like}, 74.6%; *bla*_{OXA-24/40-like}, 47.6%; and *bla*_{OXA-235-like}, 12.7%. *ISAb1*, *ISAb1-bla*_{OXA-23-like} and *ISAb1-bla*_{OXA-51-like} were detected in 100%, 41.3% and 1.6% of CRAB isolates, respectively. Co-occurrence of *bla*_{OXA} determinants or inserted *ISAb1* upstream of the corresponding genes was associated with increased carbapenem MICs (≥ 128 $\mu\text{g/mL}$).

Conclusion: The emergence of high-level CRAB with *bla*_{OXA} and *ISAb1-bla*_{OXA} family in burn patients is a matter of increasing clinical concern, emphasising the need for infection control efforts to limit such problematic bacteria.

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1. Introduction

Acinetobacter baumannii is an opportunistic pathogen increasingly known to cause serious nosocomial infections, especially in immunocompromised patients with prolonged hospitalisation [1,2]. It is well established that *A. baumannii* is now designated as a 'red alert' human pathogen because of its capacity to resist

multiple classes of antimicrobial agents, including carbapenems, a group of antibiotics that have until now been used as first-line treatment against multidrug-resistant *A. baumannii* [3–5]. Carbapenem resistance may be due to the presence of intrinsic or acquired *bla*_{OXA} carbapenemase genes [6]. Production of Ambler class D β -lactamases, also known as oxacillinases, is considered as the paradigm of resistance to carbapenems in *A. baumannii* strains [7,8]. Carbapenem-hydrolysing OXA-type carbapenemases are more prevalent compared with other β -lactamases in *A. baumannii* [9]. Four major groups of OXA-type carbapenemases, including OXA-23-like, OXA-24/40-like, OXA-51-like and OXA-58-like, have been reported to play an important role in carbapenem resistance

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in *A. baumannii*. Nevertheless, other OXA types, such as OXA-143-like and OXA-235-like, have also been identified as rare enzymes worldwide [6].

Insertion sequence (IS) elements are the least complex type of transposable elements that play an important role in bacterial acquisition of antimicrobial resistance genes, and *bla*_{OXA} carbapenemase genes may be governed by these mobile genetic elements, especially *ISAbA1* [8]. *ISAbA1* belongs to the IS4 family and has 11-bp inverted repeat sequences flanked by 9-bp direct repeats of the target sequence [7]. Presence of the *ISAbA1* promoter sequence associated with *bla*_{OXA} genes strongly contributes to carbapenem resistance in *A. baumannii* [9,10]. In particular, OXA-51-like is intrinsic to *A. baumannii* and naturally exists in all strains, although it can be overexpressed when flanked by IS elements [11].

In this study, the antimicrobial susceptibility patterns of clinical *A. baumannii* isolates were determined and the different *bla*_{OXA} genes were characterised. The presence of *ISAbA1* sequence upstream of the corresponding determinants in carbapenem-resistant *A. baumannii* (CRAB) strains was also analysed.

2. Materials and methods

2.1. Bacterial isolates and microbiological methods

A total of 65 non-duplicate *A. baumannii* isolates were recovered during 2013 from patients hospitalised at Shahid Motahari Burn Center in Tehran, Iran. The hospital currently works as a subset of Iran University of Medical Sciences (IUMS) and is a large referral centre providing tertiary health care to burn patients from throughout Iran. All isolates were initially designated as *A. baumannii* on the basis of standard biochemical tests and were subsequently confirmed by PCR amplification of the *bla*_{OXA-51-like} gene and the 16S–23S rRNA internal transcribed spacer (ITS) region [12,13].

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) procedures [14] and breakpoint interpretations for 12 antimicrobial agents, including ceftazidime (30 µg), cefepime (30 µg), piperacillin/tazobactam (100/10 µg), ampicillin/sulbactam (10/10 µg), imipenem (10 µg), meropenem (10 µg),

gentamicin (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), tetracycline (30 µg) and minocycline (30 µg) (Mast Group Ltd., Bootle, UK). The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) of CRAB isolates to imipenem, meropenem, polymyxin B, colistin and tigecycline (Sigma-Aldrich, St Louis, MO) according to CLSI guidelines. Since there are no interpretive breakpoints for tigecycline in the CLSI guidelines, the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for *Enterobacteriaceae* were applied (MICs of ≤1 µg/mL and >2 µg/mL were defined as susceptible and resistant, respectively) [15]. *A. baumannii* isolates were categorised as multidrug-resistant (MDR), extensively drug-resistant (XDR) or pandrug-resistant (PDR) according to the definition provided by Magiorakos et al. [16]. *Pseudomonas aeruginosa* ATCC[®] 27853 and *Escherichia coli* ATCC[®] 25922 were used as control strains.

2.3. PCR and DNA sequencing

Genomic DNA from the prepared *A. baumannii* isolates was extracted by the boiling method. The PCR primers specific for the genes encoding OXA-23-like, OXA-24/40-like, OXA-51-like, OXA-58-like, OXA-143-like and OXA-235-like as well as *ISAbA1* element are listed in Table 1. Multiplex PCR was carried out on a Mastercycler[®] PeQ STAR Gradient thermal cycler (PeQLab Biotechnologie GmbH, Erlangen Germany) using Taq DNA Polymerase 2× Master Mix RED (Ampliqon A/S, Odense, Denmark) in a total volume of 25 µL containing 20 ng of template DNA and 10 pmol/µL of each forward and reverse primer. PCR cycling conditions were as follows: initial denaturation at 94 °C for 4 min; 35 cycles of denaturation at 94 °C for 60 s, annealing at 56 °C for 60 s and extension at 72 °C for 60 s; followed by a final extension at 72 °C for 5 min. Presence of the *ISAbA1* promoter sequence upstream of the *bla*_{OXA} genes was investigated by a series of conventional PCRs using the forward primer for *ISAbA1* and the reverse primer for *bla*_{OXA} [10]. PCR was performed under conditions exactly as for the *bla*_{OXA} genes and *ISAbA1* element, except that an annealing temperature of 52 °C for 45 s was used. In addition, a single PCR was performed to amplify *ISAbA1*–*bla*_{OXA-51-like} and *ISAbA1*–*bla*_{OXA-23-like} sequences as described previously (Table 1) [8]. PCR products were fractionated by agarose gel electrophoresis and were then visualised by SYBR[®] Safe DNA Gel Stain (Thermo Fisher Scientific, Waltham, MA) under ultraviolet light. Amplicons were sequenced

Table 1
PCR primer sequences used in this study.

Reference	Product size (bp)	Primer sequence (5' → 3')	Assay/target
Multiplex PCR for detecting <i>bla</i> _{OXA} genes and the <i>ISAbA1</i> element [8]	353	F: TAATGCTTTGATCGGCCTTG R: TGGATTGCACTTCATCTTGG	<i>bla</i> _{OXA-51-like}
[8]	599	F: AAGTATTGGGGCTTGTGCTG R: CCCCTCTGCGCTCTACATAC	<i>bla</i> _{OXA-58-like}
[8]	501	F: GATCGGATTGGAGAACCAGA R: ATTTCTGACCGCATTTCAT	<i>bla</i> _{OXA-23-like}
[8]	246	F: GGTTAGTTGGCCCCCTAAA R: AGTTGAGCGAAAAGGGGATT	<i>bla</i> _{OXA-24/40-like}
[8]	149	F: TGGCACTTCAGCAGTTCCT R: TAATCTTGAGGGGGCCAACC	<i>bla</i> _{OXA-143-like}
This study	593	F: AAATTTAAGACGGATCGCC R: CAATGATTTGAGTCGTGCAC	<i>bla</i> _{OXA-235-like}
[8]	548	F: CACGAATGCAGAAGTTG R: CGACGAATACTATGACAC	<i>ISAbA1</i>
PCR for detecting <i>ISAbA1</i> upstream of <i>bla</i> _{OXA} genes [8]	359	F: CAAGGCCGATCAAAGCATT R: GTGTCATAGTATTCGTCC	<i>ISAbA1</i> – <i>bla</i> _{OXA-51-like}
[8]	875	F: GTGTCATAGTATTCGTCC R: ATTTCTGACCGCATTTCAT	<i>ISAbA1</i> – <i>bla</i> _{OXA-23-like}

using an ABI 3730XL DNA Analyzer (Applied Biosystem Inc., Foster City, CA) using Sanger (dideoxy chain termination) technology. Nucleotide sequences were analysed using Chromas v.2.6.4 (<http://www.technelysium.com.au>) and NCBI BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Artemis v.15.0.0 (<http://www.sanger.ac.uk/science/tools/artemis>) followed by ClustalX v.2.0.12 (<http://clustalx.software.informer.com/2.0/>) was used for nucleotide alignment.

2.4. Statistical analysis

All obtained data were analysed using PASW Statistics v.18.0 (SPSS Inc., Chicago, IL). Differences between categorical variables were compared by χ^2 test and one-way analysis of variance (ANOVA). A *P*-value of <0.05 was considered statistically significant.

3. Results

3.1. Antimicrobial susceptibility profile of the *A. baumannii* isolates

The results of disk diffusion susceptibility testing for all 65 *A. baumannii* isolates showed the highest resistance rates to four antibiotics, including imipenem, meropenem, ceftazidime and piperacillin/tazobactam (96.9% each), whilst minocycline was the most active agent with 100% susceptibility. Of the 65 *A. baumannii* isolates, 63 (96.9%) were carbapenem-resistant (i.e. CRAB), whilst 2 isolates (3.1%) were carbapenem-susceptible. The prevalence of *A. baumannii* isolates meeting the clinical definition for MDR, XDR and PDR phenotypes was 96.9% (*n*=63), 72.3% (*n*=47) and 0%, respectively. Table 2 shows the MIC distributions of five antimicrobial agents against the 63 CRAB isolates. The MIC test confirmed the rate of 96.9% CRAB isolates identified by the disk diffusion method. For imipenem, 57.1% (*n*=36) and 42.9% (*n*=27) of CRAB isolates had MICs in the range 8–32 µg/mL and 64 to ≥128 µg/mL, respectively. The MIC range for meropenem was 8–32 µg/mL in 54.0% of the CRAB isolates (*n*=34), whilst 46.0% (*n*=29) had MICs of 64 to ≥128 µg/mL to this antibiotic. All CRAB isolates were susceptible to polymyxin B, whilst 34.9% (*n*=22) and 12.7% (*n*=8) exhibited resistance to tigecycline (MIC = 16 µg/mL) and colistin (MIC = 4 µg/mL), respectively.

3.2. *bla*_{OXA} resistance determinants, IS*Aba*1 element, and carbapenem resistance

All 63 CRAB isolates were subjected to PCR assay for six distinct *bla*_{OXA} genes and the IS*Aba*1 element. Intrinsic *bla*_{OXA-51-like} was detected in all 63 CRAB isolates (100%), verifying them as *A. baumannii*. The prevalence of *bla*_{OXA-23-like} and *bla*_{OXA-24/40-like} genes was 74.6% (*n*=47) and 47.6% (*n*=30), respectively. Notably, eight CRAB isolates (12.7%) were found to be positive for *bla*_{OXA-235-like}. Nucleotide sequencing analysis of the *bla*_{OXA-235-like} amplicon indicated that it is a part of Tn6252 transposon inserted into the *A. baumannii* conjugative plasmid pRCH51-3 (GenBank accession

no. KY216144). The non-redundant (nr) NCBI protein database also revealed that they have the closest match (99% identity) with OXA-134 family carbapenem-hydrolysing class D β-lactamases OXA-235-like, OXA-236-like, and OXA-237-like in *A. baumannii* (GenBank accession nos. **WP_000854009**, **WP_063862170** and **WP_000854010**, respectively). No *bla*_{OXA-58-like} and *bla*_{OXA-143-like} genes were found. The prevalence of CRAB isolates with co-occurrence of *bla*_{OXA} genes was as follows: *bla*_{OXA-51-like} + *bla*_{OXA-23-like}, 74.6% (*n*=47); *bla*_{OXA-51-like} + *bla*_{OXA-24/40-like}, 47.6% (*n*=30); *bla*_{OXA-23-like} + *bla*_{OXA-24/40-like}, 41.3% (*n*=26); and *bla*_{OXA-51-like} + *bla*_{OXA-23-like} + *bla*_{OXA-24/40-like}, 41.3% (*n*=26). All CRAB isolates carried the IS*Aba*1 element. PCR experiments to determine the association between IS*Aba*1 and six distinct *bla*_{OXA} determinants in CRAB isolates demonstrated that IS*Aba*1 was not associated with *bla*_{OXA-24/40-like} or *bla*_{OXA-235-like}, whilst 1 (1.6%) and 26 (41.3%) CRAB isolates had *bla*_{OXA-51-like} and *bla*_{OXA-23-like} genes with upstream insertion of IS*Aba*1, respectively.

The MIC distributions for imipenem and meropenem and the *bla*_{OXA} carbapenemase genes in CRAB isolates are shown in Table 3. Generally, the majority of CRAB isolates with *bla*_{OXA-24/40-like} (*n*=30) showed significantly higher MICs to carbapenems compared with those harbouring other single *bla*_{OXA} genes (*P*<0.001). For imipenem, 9/30 isolates (30.0%) had MICs in the range 8–32 µg/mL, whilst 21/30 (70.0%) exhibited MICs of 64–128 µg/mL. Similarly, the meropenem MIC range was 8–32 µg/mL and 64 to >128 µg/mL for 8/30 (26.7%) and 22/30 (73.3%) isolates, respectively. Conversely, there was no significant difference in MICs to carbapenems among the isolates that carried other distinct resistance determinants (*P*>0.09). Furthermore, CRAB isolates with the concomitant existence of several *bla*_{OXA} determinants or inserted IS*Aba*1 near these genes generally had a high level of resistance to carbapenems. In fact, in all *bla*_{OXA} co-occurrence groups except *bla*_{OXA-51-like} + *bla*_{OXA-23-like}, the majority of isolates showed high carbapenem MICs (64 to >128 µg/mL). Similarly, one IS*Aba*1-*bla*_{OXA-51-like}-carrying isolate was highly resistant to both imipenem and meropenem with MICs of >128 µg/mL to each. Of 26 IS*Aba*1-*bla*_{OXA-23-like}-carrying isolates, 15 (57.7%) and 13 (50.0%) were classified as highly resistant to imipenem and meropenem (MICs ≥ 128 µg/mL to each), respectively.

3.3. Nucleotide sequence analysis

A selection of the *bla*_{OXA} nucleotide sequences reported in the present study have been submitted to the NCBI GenBank database under accession nos. MG283235, MG283236, MG859906, MG859907, MG920242, MH191363, MH212134 and MG920244.

4. Discussion

For the effective treatment and management of MDR *A. baumannii* infections it is necessary to determine regional susceptibility patterns as well as molecular epidemiological data such as the *bla*_{OXA} gene distribution of CRAB isolates. This is the

Table 2

Minimum inhibitory concentration (MIC) distribution of five antimicrobial agents against 63 carbapenem-resistant *Acinetobacter baumannii* isolates.

Antibiotic	MIC range (µg/mL)	No. (%) of isolates with MIC (µg/mL) at:											
		<0.25	0.25	0.5	1	2	4	8	16	32	64	128	>128
Imipenem	8 to >128	0	0	0	0	0	0	7 (11.1)	16 (25.4)	13 (20.6)	11 (17.5)	8 (12.7)	8 (12.7)
Meropenem	8 to >128	0	0	0	0	0	0	1 (1.6)	19 (30.2)	14 (22.2)	13 (20.6)	9 (14.3)	7 (11.1)
Polymyxin B	0.25–2	0	9 (14.3)	16 (25.4)	24 (38.1)	14 (22.2)	0	0	0	0	0	0	0
Colistin	<0.25–4	1 (1.6)	3 (4.8)	13 (20.6)	12 (19.0)	26 (41.3)	8 (12.7)	0	0	0	0	0	0
Tigecycline	<0.25–16	7 (11.1)	6 (9.5)	8 (12.7)	19 (30.2)	1 (1.6)	0	0	22 (34.9)	0	0	0	0

Table 3Association between the distribution of *bla*_{OXA} genes and the IS*Aba1* insertion sequence element in 63 carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates.

Meropenem			Imipenem			No. (%) of CRAB isolates	Resistance determinant
P-value	MIC (µg/mL)	No. (%) of resistant isolates	P-value	MIC (µg/mL)	No. (%) of resistant isolates		
0.09	8	1 (1.6)	0.1	8	7 (11.1)	63 (100)	<i>bla</i> _{OXA-51} -like
	16	19 (30.2)		16	16 (25.4)		
	32	14 (22.2)		32	13 (20.6)		
	64	13 (20.6)		64	11 (17.5)		
	128	9 (14.3)		128	8 (12.7)		
0.09	>128	7 (11.1)	0.1	>128	8 (12.7)	47 (74.6)	<i>bla</i> _{OXA-23} -like
	8	0 (0)		8	3 (6.4)		
	16	10 (21.3)		16	8 (17.0)		
	32	12 (25.5)		32	11 (23.4)		
	64	11 (23.4)		64	10 (21.3)		
0.1	128	7 (14.9)	0.2	128	8 (17.0)	30 (47.6)	<i>bla</i> _{OXA-24/40} -like
	>128	7 (14.9)		>128	7 (14.9)		
	8	1 (3.3)		8	1 (3.3)		
	16	2 (6.7)		16	4 (13.3)		
	32	5 (16.7)		32	4 (13.3)		
0.3	64	11 (36.7)	0.3	64	10 (33.3)	8 (12.7)	<i>bla</i> _{OXA-235} -like
	128	7 (23.3)		128	8 (26.7)		
	>128	4 (13.3)		>128	3 (10.0)		
	8	0 (0)		8	1 (12.5)		
	16	3 (37.5)		16	5 (62.5)		
0.09	32	5 (62.5)	0.1	32	2 (25.0)	47 (74.6)	<i>bla</i> _{OXA-51} -like + <i>bla</i> _{OXA-23} -like
	64	13 (27.7)		64	10 (21.3)		
	128	9 (19.1)		128	8 (17.0)		
	>128	7 (14.9)		>128	8 (17.0)		
	8	0 (0)		8	3 (6.4)		
0.09	16	6 (12.8)	0.1	16	8 (17.0)	30 (47.6)	<i>bla</i> _{OXA-51} -like + <i>bla</i> _{OXA-24/40} -like
	32	12 (25.5)		32	10 (21.3)		
	64	13 (27.7)		64	10 (21.3)		
	128	9 (19.1)		128	8 (17.0)		
	>128	7 (14.9)		>128	8 (17.0)		
0.09	8	1 (3.3)	0.1	8	1 (3.3)	26 (41.3)	<i>bla</i> _{OXA-23} -like + <i>bla</i> _{OXA-24/40} -like
	16	4 (13.3)		16	3 (10.0)		
	32	4 (13.3)		32	4 (13.3)		
	64	8 (26.7)		64	9 (30.0)		
	128	7 (23.3)		128	7 (23.3)		
0.1	>128	6 (20.0)	0.1	>128	6 (20.0)	26 (41.3)	<i>bla</i> _{OXA-23} -like + <i>bla</i> _{OXA-24/40} -like
	8	0 (0)		8	1 (3.8)		
	16	2 (7.7)		16	1 (3.8)		
	32	2 (7.7)		32	2 (7.7)		
	64	11 (42.3)		64	7 (26.9)		
0.1	128	7 (26.9)	0.1	128	8 (30.8)	26 (41.3)	<i>bla</i> _{OXA-51} -like + <i>bla</i> _{OXA-23} -like + <i>bla</i> _{OXA-24/40} -like
	>128	4 (15.4)		>128	7 (26.9)		
	8	1 (3.8)		8	1 (3.8)		
	16	1 (3.8)		16	1 (3.8)		
	32	1 (3.8)		32	2 (7.7)		
ND	64	7 (26.9)	ND	64	6 (23.1)	1 (1.6)	IS <i>Aba1</i> - <i>bla</i> _{OXA-51} -like
	128	9 (34.6)		128	8 (30.8)		
	>128	7 (26.9)		>128	8 (30.8)		
	>128	1 (100)		>128	1 (100)		
	8	0 (0)		8	3 (11.5)		
0.2	16	2 (7.7)	0.2	16	1 (3.8)	26 (41.3)	IS <i>Aba1</i> - <i>bla</i> _{OXA-23} -like
	32	3 (11.5)		32	3 (11.5)		
	64	8 (30.8)		64	4 (15.4)		
	128	6 (23.1)		128	8 (30.8)		
	>128	7 (26.9)		>128	7 (26.9)		

MIC, minimum inhibitory concentration; ND, not determined.

first epidemiological study reporting the detection of *bla*_{OXA-235}-like-harboring CRAB isolates from Iran.

Consistent with the findings of other similar studies [3,5,9], a significant percentage of *A. baumannii* isolated in this study showed a MDR or XDR phenotype. In addition, the high rate of resistance to carbapenems, as reported in other studies [5,17], is a cause of great concern, making the treatment of *A. baumannii* infections difficult. In contrast, polymyxins, especially polymyxin B, showed excellent activity against CRAB isolates, in agreement with previous studies [3,4]. These findings indicate that polymyxins have increasingly become the last-resort treatment for MDR *A. baumannii* infections. However, there is a concern that overuse of polymyxins creates an opportunity for the emergence of bacterial resistance, threatening the efficacy of these antibiotics.

This is supported by the detection of polymyxin-resistant isolates in our therapeutic centre, strongly emphasising the need for a significant effort to maintain the antibacterial potential of these polycationic agents. Furthermore, tigecycline exhibited reasonable in vitro activity against CRAB isolates. However, observed resistance to tigecycline, albeit at a low frequency, is particularly worrying and should be studied more, although this drug has not yet become commercially available in Iran [3].

There are several reports of *A. baumannii* carrying different types of *bla*_{OXA} genes from Iran [17,18]. However, this is the first report of the detection of the *bla*_{OXA-235}-like gene among CRAB isolates. There are generally not enough data on the geographical distribution of the *bla*_{OXA-235}-like gene. Higgins et al. detected for the first time a novel OXA-type carbapenemase (OXA-235) in eight

A. baumannii originating from the USA [19]. Since OXA-235-like shows high affinity for carbapenems, it may play a role in carbapenem resistance in *A. baumannii* [19], as *bla*_{OXA-235-like}-carrying *A. baumannii* isolates in the present study showed rather high resistance to both imipenem (MICs of 8–32 µg/mL) and meropenem (MICs of 16–32 µg/mL).

This study revealed that positivity for a distinct oxacillinase-encoding determinant was not definitely related to increased resistance to carbapenems in itself. Instead, co-occurrence of these genes was a source of high-level resistance (MICs of 64 to >128 µg/mL) in *A. baumannii*, as observed in previous studies [1,20]. In particular, in Egypt, Al-Hassan et al. found that resistance to imipenem and meropenem (MICs ≥ 8 µg/mL) was correlated with the presence of the *bla*_{OXA-23-like}, *bla*_{OXA-58-like} and *bla*_{OXA-24/40-like}, whilst carbapenem-susceptible isolates harboured only *bla*_{OXA-51-like} [1].

The results of the present study are in agreement with those of previous studies showing that *ISAbal1* promotes the expression of the antimicrobial resistance genes *bla*_{OXA-51-like} and *bla*_{OXA-23-like} [9,11]. Similar to that observed for the co-existence of *bla*_{OXA} genes, it was found that one *ISAbal1*-*bla*_{OXA-51-like}-carrying isolate and a high proportion of *ISAbal1*-*bla*_{OXA-23-like}-carrying isolates were classified as highly carbapenem-resistant. These findings on the distribution of *bla*_{OXA-23-like} adjacent to *ISAbal1* in Iranian *A. baumannii* isolates indicate plasmid-borne transfer among the species. However, it is unsure whether OXA-type enzymes have adequate activity to contribute to carbapenem resistance in the absence of *ISAbal1*. Further studies, such as quantitative real-time PCR, are needed to confirm the role of *ISAbal1* in the overproduction of oxacillinases encoded by *bla*_{OXA} genes.

In conclusion, the presence of *bla*_{OXA} determinants and the *ISAbal1* element upstream of the corresponding genes among multiresistant CRAB could be responsible for carbapenem resistance. The emergence of oxacillinase-producing strains in burn patients is a matter of increasing clinical concern in Iran, highlighting the necessity to monitor resistance levels and mechanisms of resistance among *A. baumannii* strains.

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Competing interests

None declared.

Ethical approval

Not required.

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