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RESEARCH ARTICLE

Association of carbapenem and multidrug resistance with the expression of efflux pump-encoding genes in *Pseudomonas aeruginosa* clinical isolates

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ABSTRACT

Efflux pumps play an important role in the emergence of antibiotic-resistant *Pseudomonas aeruginosa* strains. The present study aimed to assess the expression of the MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM efflux pumps in carbapenem-resistant and multidrug-resistant (MDR) *P. aeruginosa* strains isolated from clinical specimens between June 2019 and January 2022 in Ardabil city. The presence of efflux pump-encoding genes, *i.e. mexA*, *mexC*, *mexE*, and *mexY*, was assessed using the polymerase chain reaction (PCR) technique in 48 carbapenem-resistant and MDR *P. aeruginosa* strains. Real-time reverse transcription PCR was employed to evaluate the expression levels of *mexA*, *mexC*, *mexE*, and *mexY* genes. All 48 carbapenem-resistant and MDR *P. aeruginosa* strains harbored efflux pump-encoding genes including *mexA*, *mexC*, *mexE*, and *mexY* according to the PCR results. Overexpression of the MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM efflux pumps was detected in 75% ($n = 36$), 83.3% ($n = 40$), 10.4% ($n = 5$) and 41.6% ($n = 20$) of the clinical isolates of *P. aeruginosa,* respectively. This study revealed that the presence and overexpression of efflux pumps are associated with the emergence of carbapenem-resistant and MDR *P. aeruginosa* strains. Therefore, research on efflux pump inhibitors of *P. aeruginosa* will be a worthwhile endeavor to increase the clinical efficiency of available antibiotics and prevent ensuing treatment failure.

KEYWORDS

Pseudomonas aeruginosa, efflux pump, carbapenem, multidrug resistance

INTRODUCTION

Since the mid-twentieth century when antibiotics were introduced to the market, the emergence of drug resistance has threatened the treatment efficacy of bacterial infection, which remains a significant global health concern [1]. Irrational antibiotic usage is regarded as the most important reason for the emergence of antibiotic resistance in pathogenic bacterial populations [2]. Accordingly, the World Health Organization (WHO) in 2017 introduced antibiotic-resistant bacteria as a global threat to human health, for which there is an urgent need to develop new antibiotics [3]. One of these bacteria is carbapenem-resistant *Pseudomonas aeruginosa* [3]. Hence, knowledge about the prevalence and drug resistance

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mechanisms will be useful to control this microorganism and may help select appropriate empirical therapy. The resistance of *P. aeruginosa* strains to carbapenem antibiotics involves intrinsic, acquired, or adaptive nature and is mediated by carbapenemases, AmpC cephalosporinase, OprD porin, and efflux pumps [4, 5]. Efflux pumps (Mex pumps) can extrude one or more antibiotics from bacterial cells into the external environment and hence are involved in intrinsic and acquired resistance as well as the emergence of multidrug-resistant (MDR) *P. aeruginosa* strains, which are considered a growing threat worldwide, similar to carbapenem-resistant strains [1, 6]. In addition, these pumps can repel various disinfectants, detergents, and dyes [7]. Only five out of 12 known types of multidrug efflux pumps identified in *P. aeruginosa* strains are involved in antibiotic resistance in the clinical setting [1]. This three-component RND (the resistance-nodulation cell division) family transporters with different substrate specificities include MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM, and MexJK-OprM [1]. Among them, the constitutively expressed MexAB-OprM and drug-inducible MexXY-OprM efflux pumps are responsible for the intrinsic resistance of *P. aeruginosa* to many antibiotics, while other pumps are expressed under specific conditions and contribute to acquired antibiotic resistance [1, 8, 9]. Overexpression of *P. aeruginosa* Mex pumps might cause challenges against successful infection treatment [7]. To assess the role of efflux pumps in the emergence of *P. aeruginosa* strains resistant to different antibiotics in Ardabil hospitals, we evaluated the expression of the chromosomally encoded genes MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM in carbapenem-resistant as well as MDR *P. aeruginosa* strains isolated from clinical specimens.

MATERIALS AND METHODS

Bacterial strains, materials, and equipment

A total of 48 *P. aeruginosa* strains were studied in the present study, which were collected from Ardabil hospitals between June 2019 and January 2022. The bacterial strains were MDR and resistant to imipenem. Bacterial identification and confirmation as well as antimicrobial susceptibility were previously determined using standard laboratory tests and disk diffusion method, respectively [5, 10]. In addition, characteristics of carbapenem resistance mechanisms other than efflux pump-associated ones were previously assessed [11]. In this study, SYBR Green PCR Master Mix without ROX™ and PCR master mix were obtained from Ampliqon, Denmark. Primers were synthesized by Metabion, Germany. RNA extraction kit and cDNA synthesis kit were purchased from Favorgen, Taiwan, and YTA, Iran, respectively. In addition, Eppendorf thermal cycler, Germany, for gene amplification and LightCycler® System, Roche Diagnostics, for mRNA transcription were used. The quality and quantity of extracted DNAs and RNAs were confirmed by NanoDrop 2000c Spectrophotometer (Thermo Scientific, USA).

Polymerase chain reaction (PCR)

Detection of the efflux pumps encoding genes, *i.e. mexA*, *mexC*, *mexE*, and *mexY*, in 48 carbapenem-resistant and MDR *P. aeruginosa* clinical isolates was performed using the PCR technique. The total DNA of resistant *P. aeruginosa* strains was extracted using the boiling method and then stored at −20 °C temperature. According to the PCR programs and specific primers presented in Table 1, gene amplifications were done in a total volume of 20 µL containing 15 µL of PCR master mix, 2 µL of primers (10 µmol L^{-1}), and 3 µL of template DNA. Amplified genes were visualized on 1% agarose gel using the electrophoresis technique and then confirmed using the sequencing method.

Real-time reverse transcription PCR (real-time RT-PCR)

The expression of mRNA for the *mexA*, *mexC*, *mexE*, and *mexY* genes was performed in 48 carbapenem-resistant and MDR *P. aeruginosa* using the real-time RT-PCR technique. For this purpose, total RNA extraction and cDNA synthesis were done according to the manufacturer's instructions. The expression of efflux pump-encoding genes was performed based on the real-time RT-PCR condition presented in

Table 1. Oligonucleotide sequences of the primers used in this study along with PCR and real-time RT-PCR programs

Gene	Oligonucleotide sequence (5′ to 3′)	PCR condition	Amplicon size (bp)	Real-time RT-PCR condition	Reference
mexA	F: CCTGCTGGTCGCGATTTCGG R: CCAGCAGCTTGTAGCGCTGG	Initial denaturation at 95 °C for 5 min (1 cycle), Denaturation at 94 °C for 1 min Annealing at 64 °C for 30 sec (34 cycles) Extension at 72 °C for 1 min	332	Pre-incubation at 95 °C for 600 s (1 cycle) Amplification: 95 °C for 20 s; 64 °C for 20 s (40 cycles); 72 °C for 30 s	[13]
mexC	F: TTGGCTATGGCCATCGCGTT R: ATCGAAGTCCTGCTGGCTGA	Initial denaturation at 95 °C for 5 min (1 cycle), Denaturation at 94 °C for 1 min Annealing at 59 °C for 30 sec (34 cycles) Extension at 72 °C for 1 min	390	Pre-incubation at 95 °C for 600 s (1 cycle) Amplification: 95 °C for 20 s; 59 °C for 20 s (40 cycles); 72 °C for 30 s	[13]
mexE	F: ATCCCACTTCTCCTGGCGCT R: GGTCGCCTTTCTTCACCAGT	Initial denaturation at 95 °C for 5 min (1 cycle), Denaturation at 94 °C for 1 min Annealing at 59 °C for 30 sec (34 cycles) Extension at 72 °C for 1 min	260	Pre-incubation at 95 °C for 600 s (1 cycle) Amplification: 95 °C for 20 s; 59 °C for 20 s (40 cycles); 72 °C for 30 s	[13]
mexY	F: CCGCTACAACGGCTATCCCT R: AGCGGGATCGACCAGCTTTC	Initial denaturation at 95 °C for 5 min (1 cycle), Denaturation at 94 °C for 1 min Annealing at 62 °C for 30 sec (34 cycles) Extension at 72 °C for 1 min	246	Pre-incubation at 95 °C for 600 s (1 cycle) Amplification: 95 °C for 20 s; 62 °C for 20 s (40 cycles); 72 °C for 30 s	[14]
rpsL	F: GCTGCAAAACTGCCCGCAACG R: ACCGCAGGTGTCCAGCGAACC		250	Pre-incubation at 95 °C for 600 s (1 cycle) Amplification: 95 °C for 20 s; 64 °C for 20 s (40 cycles); 72 °C for 30 s	[15]

Table 1 and in a total volume of 15 µL containing 7 µL of SYBR Green PCR Master Mix, 2 µL of primers (10 µmol L^{-1}), 1 µL of cDNA, and 5 µL of DEPC-treated water. All reactions were performed in duplicate and the results were checked in terms of nonspecific amplification on 1% agarose gel. The expression of mRNA in clinical isolates was normalized to the housekeeping gene *rpsL* and then compared to *P. aeruginosa* ATCC 27853 using the $2^{-\Delta\Delta Ct}$ method. The interpretation of the results was as follows: an increase of at least 2-fold for the *mexA* gene in clinical isolates, 2-fold for the *mexC* gene, 10-fold for the *mexE* gene, and 4-fold for the *mexY* gene were considered as overexpression [12]. The primers used for PCR and real-time RT-PCR are given in Table 1. A carbapenem-sensitive *P. aeruginosa* clinical isolate (strain No. 148) was used as a negative control.

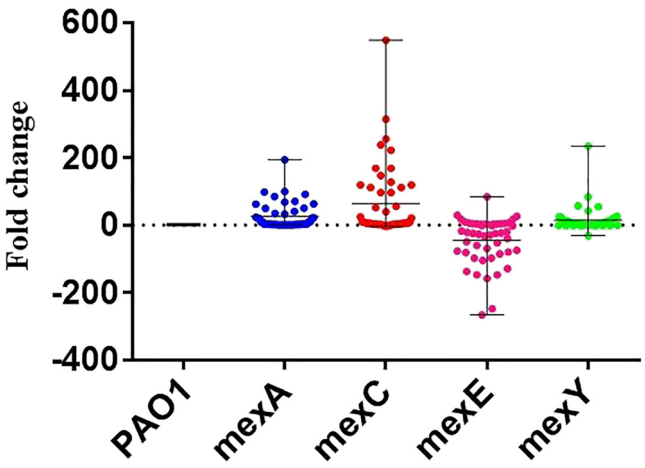

Fig. 1. Expression rates of efflux pump genes among imipenem-resistant and MDR *P. aeruginosa* clinical isolates compared with the reference strain PAO1. Each colored circle represents a strain

RESULTS

All 48 imipenem-resistant and MDR clinical isolates of *P. aeruginosa* were found to carry efflux pump-encoding genes *mexA*, *mexC*, *mexE*, and *mexY* according to the PCR results. The accession numbers for the detected *mexA*, *mexC*, *mexE*, and *mexY* genes in the GenBank database are ON920994 to ON920997.

Based on the real-time RT-PCR results, efflux pump expression status in imipenem-resistant and MDR *P. aeruginosa* was as follows: MexAB-OprM overexpression was observed in 75% (n = 36) of the isolates. As shown in Table 2, the highest frequency of gene expression of the MexAB-OprM efflux pump was observed in imipenem- and ofloxacin-resistant *P. aeruginosa* strains (33 strains). As shown in Fig. 1, MDR strain no. 130 showed the highest increase in *mexA* gene expression compared to the standard strain of *P. aeruginosa* (194-fold). The range of *mexA* gene overexpression varied from 2- to 194-fold.

The frequency of MexCD-OprJ overexpression was 83.3% (n = 40). Similar to MexAB-OprM, the highest frequency of gene expression of the MexCD-OprJ efflux pump was observed in imipenem- and ofloxacin-resistant *P. aeruginosa* strains (37 and 36 strains, respectively). MDR strain no. 75 indicated the highest increase in *mexC* gene expression compared to the standard strain of *P. aeruginosa* (548.7-fold) (Fig. 1). The range of *mexC* gene overexpression varied from 2- to 548.7-fold.

MexEF-OprN overexpression was detected in 10.4% (n = 5) of isolates and imipenem-resistant *P. aeruginosa* strains (5 strains) indicated the highest frequency of gene

Table 2. Frequency of efflux pump gene expression in 48 imipenem-resistant and MDR *P. aeruginosa* based on the resistance to different antibiotics

Antibiotics	Resistance rate (n)	Efflux pumps (n) (%)			
		MexAB-OprM	MexCD-OprJ	MexEF-OprN	MexXY-OprM
Piperacillin	30	22 (73.3)	23 (76.6)	3 (10)	13 (43.3)
Piperacillin-tazobactam	22	17 (77.2)	16 (72.7)	3 (13.6)	10 (45.4)
Ticarcillin-clavulanate	25	20 (80)	19 (76)	3 (12)	14 (56)
Ceftazidime	32	22 (68.7)	22 (68.7)	1 (3.1)	11 (34.3)
Cefepime	37	25 (67.5)	26 (70.2)	2 (5.4)	14 (37.8)
Aztreonam	7	5 (71.4)	5 (71.4)	0	3 (42.8)
Doripenem	11	9 (81.8)	6 (54.5)	1 (9)	7 (63.6)
Imipenem	48	33 (68.7)	37 (77)	5 (10.4)	18 (37.5)
Meropenem	37	24 (64.8)	28 (75.6)	2 (5.4)	10 (27)
Gentamicin	36	27 (75)	29 (80.5)	3 (8.3)	15 (41.6)
Tobramycin	37	24 (64.8)	28 (75.6)	3 (8.1)	13 (35.1)
Amikacin	34	24 (70.5)	28 (82.3)	1 (2.9)	12 (35.2)
Netilmicin	16	12 (75)	10 (62.5)	2 (12.5)	8 (50)
Ciprofloxacin	42	31 (73.8)	33 (78.5)	2 (4.7)	16 (38)
Levofloxacin	42	30 (71.4)	33 (78.5)	2 (4.7)	15 (35.7)
Lomefloxacin	25	19 (76)	16 (64)	2 (8)	13 (52)
Ofloxacin	46	33 (71.7)	36 (78.2)	3 (6.5)	18 (39.1)
Norfloxacin	41	30 (73.1)	33 (80.4)	1 (2.4)	15 (36.5)

expression. MDR strain no. 130 showed the highest increase in *mexE* gene expression (84.4-fold). The range of *mexE* gene overexpression varied from 16.56- to 84.4-fold.

Imipenem- and ofloxacin-resistant *P. aeruginosa* strains (18 strains) showed the highest frequency of gene expression MexXY-OprM. Among them, MDR strain no. 140 showed the highest increase in *mexY* gene expression (234-fold). The range of *mexY* gene overexpression varied from 5- to 234-fold. The total MexXY-OprM overexpression rate was 41.6% (n = 20).

Based on Table 3, 9 out of 48 (18.75%) imipenem-resistant *P. aeruginosa* clinical isolates had all four resistance mechanisms to carbapenems. Additionally, 47.9% (n = 23) of imipenem-resistant *P. aeruginosa* strains were carrying carbapenemases encoding genes (*i.e. IMP*, *VIM*, *OXA-23*, and *OXA-48*). Downregulation of OprD porin was observed

Table 3. Different carbapenem resistance mechanisms among 48 imipenem-resistant and MDR *P. aeruginosa* clinical isolates

Isolate number	Resistance mechanisms			
29	-	AmpC cephalosporinase	OprD porin	*mexA, mexC, mexY*
32	-	AmpC cephalosporinase	OprD porin	*mexA, mexC, mexY*
34	Carbapenemase (bla_{IMP})	-	OprD porin	*mexA, mexC*
36	-	AmpC cephalosporinase	-	*mexA, mexC, mexE, mexY*
39	Carbapenemase (bla_{IMP})	-	-	*mexA, mexC, mexE, mexY*
40	-	AmpC cephalosporinase	OprD porin	*mexA*
41	Carbapenemase (bla_{VIM})	AmpC cephalosporinase	OprD porin	*mexA, mexC, mexE, mexY*
43	Carbapenemase (bla_{IMP})	AmpC cephalosporinase	OprD porin	*mexA, mexC, mexY*
49	-	-	OprD porin	*mexA, mexY*
61	-	-	OprD porin	*mexA*
62	Carbapenemase (bla_{OXA-23})	AmpC cephalosporinase	OprD porin	*mexA, mexC, mexY*
63	Carbapenemase (bla_{OXA-23})	AmpC cephalosporinase	OprD porin	*mexA, mexC, mexY*
64	-	-	OprD porin	*mexA, mexC, mexY*
65	Carbapenemase (bla_{IMP}, bla_{OXA-23})	AmpC cephalosporinase	OprD porin	*mexA, mexC, mexY*
66	Carbapenemase (bla_{IMP})	-	OprD porin	-
68	Carbapenemase (bla_{OXA-23})	-	OprD porin	*mexC*
72	-	-	OprD porin	*mexC*
74	Carbapenemase (bla_{OXA-23})	-	OprD porin	*mexA, mexC*
75	Carbapenemase (bla_{OXA-23})	-	-	*mexA, mexC, mexY*
77	Carbapenemase (bla_{OXA-23})	-	OprD porin	-
79	Carbapenemase (bla_{OXA-23})	-	OprD porin	*mexA, mexC, mexY*
80	Carbapenemase (bla_{OXA-23})	-	OprD porin	*mexA, mexC, mexY*
81	Carbapenemase (bla_{OXA-23})	AmpC cephalosporinase	OprD porin	*mexA, mexC*
83	Carbapenemase (bla_{OXA-23})	-	OprD porin	*mexC*
84	Carbapenemase (bla_{OXA-23})	-	OprD porin	*mexA, mexC*
85	Carbapenemase (bla$_{OXA-23}$, bla_{OXA-48})	-	OprD porin	*mexA, mexC*
94	Carbapenemase (bla_{OXA-23})	AmpC cephalosporinase	OprD porin	*mexA, mexC*
96	Carbapenemase (bla_{OXA-23})	-	OprD porin	*mexA, mexC*
97	Carbapenemase (bla_{OXA-23})	AmpC cephalosporinase	OprD porin	*mexA, mexC, mexY*
98	Carbapenemase (bla_{OXA-23}, bla_{OXA-48})	AmpC cephalosporinase	OprD porin	*mexA, mexC, mexY*
100	Carbapenemase (bla_{OXA-23})	-	OprD porin	*mexA, mexC*
102	-	-	OprD porin	-
103	-	AmpC cephalosporinase	OprD porin	*mexA, mexC, mexY*
110	-	-	OprD porin	-
118	-	-	OprD porin	*mexA, mexC*
120	-	-	OprD porin	*mexC*
124	-	-	OprD porin	*mexA, mexC*
126	-	-	OprD porin	*mexA, mexC*
127	-	AmpC cephalosporinase	-	*mexA, mexC, mexY*
130	-	AmpC cephalosporinase	-	*mexA, mexC, mexE, mexY*
137	-	-	OprD porin	*mexA, mexC, mexE*
138	-	-	OprD porin	*mexC*
140	-	-	OprD porin	*mexY*
141	-	-	OprD porin	*mexA, mexC*
142	-	-	OprD porin	*mexA, mexC*
144	-	-	OprD porin	*mexC*
146	-	-	OprD porin	*mexA, mexC*
147	-	-	OprD porin	*mexC*
148*	-	-	-	-

*Strain No. 148 was imipenem-sensitive and non-MDR.

in 79.1% (n = 38) of imipenem-resistant *P. aeruginosa* strains. An increase in the expression of the AmpC cephalosporinase was seen in 33.3% (n = 16) of the isolates. Furthermore, 44 of 48 (91.6%) imipenem-resistant *P. aeruginosa* strains indicated overexpression of at least one of the efflux pumps.

DISCUSSION

Fluoroquinolones, aminoglycosides, and β-lactams are the most prevalent antibiotics used for the treatment of *P. aeruginosa* infections [16]. *P. aeruginosa* resistance to fluoroquinolones is mediated by amino acid alterations in the GyrA subunit of topoisomerase II (DNA gyrase) and the ParC subunit of topoisomerase IV enzymes as well as by efflux pumps [16, 17]. Our previous study showed that Thr83Ile and Asp87Asn as well as Ser87Leu and Ser87Trp were the most common amino acid alterations in the GyrA and ParC subunits, respectively, in ciprofloxacin-resistant *P. aeruginosa* clinical isolates [17]. Furthermore, *P. aeruginosa* multidrug efflux pumps can expel fluoroquinolones including ciprofloxacin, levofloxacin, and norfloxacin [18]. The present study showed that MexAB-OprM and MexCD-OprJ efflux pumps in comparison with MexEF-OprN and MexXY-OprM play an important role in the emergence of fluoroquinolone-resistant *P. aeruginosa* clinical isolates (Table 2).

Efflux pumps, particularly MexXY-OprM, play a key role in *P. aeruginosa's* resistance to antibiotics that inhibit protein synthesis. MexXY-OprM efflux pump can be induced by agents that target the ribosome and is involved in inducible resistance to aminoglycosides, macrolides, tetracyclines, and chloramphenicol [18, 19]. However, our results showed that the emergence of aminoglycoside-resistant *P. aeruginosa* strains was more commonly associated with the overexpression of MexAB-OprM and MexCD-OprJ efflux pumps (Table 2). Other resistance mechanisms to aminoglycosides in *P. aeruginosa* clinical isolates, such as acquired or chromosomally encoded aminoglycoside-modifying enzymes (AMEs), are still not clear in Ardabil.

Among β-lactam antibiotics, carbapenems are routinely used in *P. aeruginosa* infection treatment, especially MDR infections. The prevalence of imipenem-resistant *P. aeruginosa* in Iran is high and has been estimated to be 54% [20]. On the other hand, such a high resistance is evident in Ardabil as well and the resistance rate to imipenem, meropenem, and doripenem in *P. aeruginosa* clinical isolates were 66.7%, 42.9%, and 33.3%, respectively [5]. There are various *P. aeruginosa* resistance mechanisms to carbapenems. MexEF-OprN confers resistance to carbapenems [19, 21] but our results showed that MexAB-OprM and MexCD-OprJ are the most important efflux pumps that expel carbapenems as well as other β-lactam antibiotics (*i.e.* penicillins and cephalosporins) from the bacteria (Table 2). Table 3 shows that other carbapenem resistance mechanisms are also involved in the emergence of imipenem-resistant *P. aeruginosa* clinical isolates from Ardabil hospitals.

A high prevalence of MDR *P. aeruginosa* has been reported in Iran (58%) [2]. Additionally, according to our previous study, the prevalence of MDR *P. aeruginosa* isolated from Ardabil hospitals was 52% [10]. Emerging MDR *P. aeruginosa* strains are a consequence of the simultaneous presence of several resistance mechanisms including β-lactamase enzymes, target mutations in enzymes involved in DNA replication, and loss of OprD porin [18]. Our studies revealed the presence of carbapenemases-encoding genes, downregulation of OprD porin, and overexpression of the AmpC cephalosporinase in 47.9%, 79.1%, and 33.3% of the MDR *P. aeruginosa* strains. Nevertheless, it has been demonstrated that the extrusion of antibiotics from bacterial cells by efflux pumps can play a more important role in treatment failure and the emergence of MDR *P. aeruginosa* [22]. In the current study, 91.6% of MDR *P. aeruginosa* strains showed overexpression in at least one of the four efflux pumps. Noteworthy, in this study, the highest expression level of efflux pumps pertained to MexCD-OprJ (83.3%) followed by MexAB-OprM (75%), MexXY-OprM (41.6%), and MexEF-OprN (10.4%). In a study conducted by Hassuna et al. in Egypt, overexpression of MexCD-OprJ (75%) and MexXY-OprM (62%) efflux pumps was predominant in *P. aeruginosa* strains [12]. Singh et al. reported MexXY-OprM as the most commonly expressed efflux pump in MDR *P. aeruginosa* strains from Canada [19]. Oh et al. reported that 17 of 20 fluoroquinolone-resistant *P. aeruginosa* strains from Sweden overexpressed at least one of the efflux pump-encoding genes, and >50% of these strains were MDR [23]. Pan et al. reported that the prevalence of MexAB-OprM efflux pump in carbapenem-resistant *P. aeruginosa* strains in China was 17.3% [24]. In the study by Pourakbari et al. from Iran, 62% of *P. aeruginosa* strains indicated an increased expression level of MexAB-OprM efflux pump genes [25].

Despite the chromosomal nature of the efflux pump-encoding genes in *P. aeruginosa*, differences in the expression of genes can be attributed to the relatively high levels of constitutive expression in some genes (intrinsic resistance) and the acquisition of regulatory mutations in the other ones (acquired resistance) [26]. We did not evaluate the association between mutations in the regulatory genes of efflux pumps, often repressor genes, with the protein expression of efflux pumps as well as the effects of efflux pump inhibitors on the minimum inhibitory concentrations of antibiotics. These were the main limitations of the current study. Considering that *P. aeruginosa* is an opportunistic pathogen associated with nosocomial infections, investigating the role of efflux pumps in bacterial resistance to biocides, such as triclosan, is recommended.

CONCLUSION

This study revealed that efflux pumps are the most common drug resistance mechanism involved in the emergence of carbapenem-resistant and MDR *P. aeruginosa* strains isolated from Ardabil hospitals. Therefore, research on inhibitors of the Mex efflux pumps in *P. aeruginosa*, alone or

in combination with antibacterial agents, will be a worthwhile endeavor to increase the clinical efficiency of available antibiotics and prevent ensuing treatment failure.

Conflict of interest: The authors declare that there is no conflict of interest.

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