Original Article

Metallo-β-lactamase-mediated resistance among clinical carbapenem-resistant *Pseudomonas aeruginosa* isolates in northern Iran: A potential threat to clinical therapeutics

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ABSTRACT

Objective: Carbapenems are effective agents to treat multidrug-resistant (MDR) strains of bacteria, including *Pseudomonas aeruginosa*. However, there is a potential threat of emergence of carbapenem-resistant *P. aeruginosa* (CRPA). The aim of this study was to determine antibiotic susceptibility patterns and metallo-beta-lactamase (MBL)-mediated resistance in clinical *P. aeruginosa* isolates. **Materials and Methods:** Different clinical specimens were subjected to conventional culture-based identification of *P. aeruginosa*. Antimicrobial susceptibility patterns and MBL production were evaluated using the Kirby-Bauer and combined double-disk synergy test methods, respectively. Multiplex polymerase chain reaction was performed to investigate the presence of the bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, and bla_{SIM} genes. **Results:** A total of 71 clinical *P. aeruginosa* isolates were recovered, of which 28.17% were identified as CRPA. The most active antibiotics were colistin and polymyxin B (92.96% susceptibility to each). A total of 35% and 50% of CRPA isolates were MDR and extensively drug-resistant (XDR), respectively. MBL activity was shown in 20% of CRPA. A total of 90%, 40%, and 5% of CRPA isolates harbored the bla_{IMP}, bla_{VIM}, and bla_{NDM} genes, respectively. No correlation was found between the MBL-encoding genes of *P. aeruginosa* and patient characteristics. **Conclusion:** Although the prevalence of CRPA in our therapeutic centers was relatively low, this rate of carbapenem resistance reflects a threat limiting treatment choices. A high prevalence of MDR/XDR phenotypes among the MBL-producer isolates suggests the need for continuous assessment of antimicrobial susceptibility and surveillance of antibiotic prescription. In addition, infection control measures are needed to prevent further dissemination of these organisms.

KEYWORDS: *Carbapenem, Metallo-β-lactamase genes, Pseudomonas aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa, a member of the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa,* and *Enterobacter* species) group of pathogens, is responsible for life-threatening nosocomial infections, especially in critically ill and immunocompromised patients and is known by potential drug resistance mechanisms [1,2]. Multidrug-resistant *P. aeruginosa* (MDR-PA) is a matter of great concern as it not only causes severe and fatal infections but also increases the length of hospital stay, resulting in increased treatment costs [3]. Carbapenems are effective antibiotics against MDR-PA infections [4]. However, their use in the management of infections is threatened by the development of carbapenem-resistant *P. aeruginosa* (CRPA) strains [4,5]. Resistance

to the carbapenems in *P. aeruginosa* is often caused by impermeability through alteration or loss of the porin OprD [6], increased expression of an efflux pump [7], or the production of class B metallo-β-lactamases (MBLs) [4].

MBLs that have been detected primarily in *P. aeruginosa* [8] are categorized in class B in the structural classification scheme and group 3 in the functional classification system [9]. Various types of MBLs, such as the IMP, VIM, SIM, SPM, GIM, and NDM families have been described in *P. aeruginosa* worldwide [4]. Class B carbapenemases can hydrolyze most β-lactam

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group antibiotics except for monobactams (aztreonam). They are inhibited *in vitro* by metal chelators, such as ethylenediaminetetraacetic acid (EDTA), while clavulanic acid has no activity against these enzymes [9].

MBL-encoding genes are frequently located in mobile or transferable genetic structures, such as plasmids, transposons and integrons, which can contribute to their spread in different bacteria, including *Acinetobacter* species and *Enterobacteriaceae* [4]. We are several years away from implementation of a therapeutic inhibitor of MBLs, and hence, their continuous spread is becoming an important challenge for antibiotic therapy [10].

The present study was performed to characterize the prevalence of carbapenem resistance, and MBL production among *P. aeruginosa* isolates from hospitalized patients in Gorgan, in northern Iran. Herein, we describe the susceptibility profiles and class B carbapenemase genes carried by the isolates, as it might help in the worldwide comparison of resistance mechanisms of MDR Gram-negative bacilli.

MATERIALS AND METHODS

Study population and species identification

This project was approved by the Ethics Committee of Golestan University of Medical Sciences with the ethical code number IR. GUMS. REC.1395.87. Informed consent was signed by all patients who were hospitalized at four therapeutic centers, including 5th Azar, Sayyad Shirazi, Ayatollah Taleghani, and Hakim Jurjani hospitals. From February 2016 to July 2017, different biological materials, including urine, respiratory tract samples, blood, eye swabs, wound swabs, ear swabs, and a heart biopsy were obtained. All specimens were cultured on MacConkey agar and nonselective blood agar media (Merck, Darmstadt, Germany). *P. aeruginosa* isolates were identified by standard microbiological and biochemical methods, such as colony morphology and pigmentation, Gram staining, oxidase, catalase, motility, indole, citrate, and oxidative-fermentative tests, and growth at 42°C. All isolates were then stored in tryptic soy broth stock (Merck, Darmstadt, Germany) with 15% glycerol at −80°C until used.

Antimicrobial susceptibility profiling

Antibiotic susceptibility profiles of all isolates were determined using the Kirby-Bauer diffusion testing method on Mueller–Hinton agar plates (Merck, Darmstadt, Germany), according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [11]. The antibiotic disks (MAST, Merseyside, UK) used were as follows: imipenem (10 μg), meropenem (10 μg), ceftazidime (30 μg), piperacillin-tazobactam (100 μg/10 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), gentamicin (10 μg), amikacin (30 μg), tobramycin (10 μg), polymyxin B (300 U), and colistin (10 μg). Quality control testing was performed using the recommended organisms, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 35218. To characterize patterns of multiple drug resistance, isolates were defined as MDR-PA, extensively drug-resistant-PA (XDR-PA), and pandrug-resistant-PA (PDR-PA) according to Magiorakos *et al.* [12] as follows: MDR-PA, if isolates were resistant to ≥1 agent in ≥3 antimicrobial groups; XDR-PA, if isolates were

resistant to ≥1 agent in all but ≤2 groups; PDR-PA, if isolates were resistant to all antimicrobials studied. Furthermore, all isolates that met criteria of resistance to any carbapenem (imipenem or meropenem) tested, as characterized by the CLSI [11], were considered CRPA.

Phenotypic detection of metallo-beta-lactamase

CRPA cultures were evaluated for MBL production using the combined double-disk synergy test (CDDST) [13]. In brief, 100 mM EDTA solution (Merck, Darmstadt, Germany) as an MBL inhibitor (iMBL) was made by dissolving 3.72 g of disodium EDTA.2H$_2$O in 100 mL of distilled water. The pH was adjusted to 8.0 by NaOH and sterilized by autoclaving. A 0.5 McFarland suspension of the organism was inoculated onto Mueller–Hinton agar plates. Two disks each of imipenem (10 μg) and ceftazidime (30 μg) antimicrobials were placed on the inoculated Mueller–Hinton plates at a distance of 2 cm. For both β-lactam agents, 8 μL of iMBL solution was directly added to one of the disks. After an overnight incubation period at 37°C, an increase in the inhibition zone ≥8 mm around the combined disk compared to that the corresponding antimicrobial disk alone was considered positive for an MBL [13]. An MBL-producing strain of *P. aeruginosa* was used as the positive control.

Detection of metallo-beta-lactamase determinants

All CRPA isolates were examined for MBL-encoding genes, including *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{NDM}$, *bla*$_{SPM}$, and *bla*$_{SIM}$, by a multiplex polymerase chain reaction (PCR) assay using specific primers [Table 1] [14]. A DNA template was prepared using the boiling method. In brief, a few colonies of an overnight culture were suspended in 200 μL of sterile distilled water. The bacterial suspension was boiled for 15 min and centrifuged at 12000 rmp for 10 min, and the supernatant was transferred to a fresh Eppendorf tube as a template. PCR amplification was performed in 25 μL reaction mixture with 2x Master Mix Red (Ampliqon, Herlev, Denmark) containing 1 × PCR buffer ([Tris-HCl, pH 8.5], [NH$_4$]$_2$S0$_4$, 4 mM MgCl$_2$, 0.2% Tween20), 0.2 U/μL *Taq* DNA polymerase, 2 mM MgCl$_2$, and 0.4 mM of each deoxynucleotide, 0.2 μM of each primer (Macrogen, Seoul, Korea), 10 ng template DNA, and sterile distilled water. PCR was performed using the following thermal cycling protocol: initial denaturation at 94°C for 10 min, followed by 36 cycles of denaturation at 94°C

Table 1: Oligonucleotide primers used to amplify metallo-β-lactamase genes

Gene	Primer (5'→3')	Amplicon size (bp)
bla$_{IMP}$	F: GGAATAGAGTGGCTTAAYTCTC	232
	R: GGTTTAAYAAAACAACCACC	
bla$_{VIM}$	F: GATGGTGTTTGGTCGCATA	390
	R: CGAATGCGCAGCACCAG	
bla$_{SPM}$	F: AAAATCTGGGTACGCAAACG	271
	R: ACATTATCCGCTGGAACAGG	
bla$_{SIM}$	F: TACAAGGGATTCGGCATCG	570
	R: TAATGGCCTGTTCCCATGTG	
bla$_{NDM}$	F: GGTTTGGCGATCTGGTTTTC	621
	R: CGGAATGGCTCATCACGATC	

for 30 s, annealing at 52°C for 40 s, extension at 72°C for 50 s and a final extension at 72°C for 5 min. PCR products were electrophoresed on 1.5% agarose gel stained with DNA safe stain (SinaClon, Tehran, Iran), then photographed under an ultraviolet transilluminator. Sequencing of the PCR amplicons was performed with anABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA), and then, the nucleotide sequences were compared with known sequences at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST/).

Data analysis

SPSS software version 18.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Categorical and continuous quantitative variables were analyzed using Pearson's Chi-squared and independent samples t-tests, respectively. Statistical significance in this study was a $P < 0.05$.

RESULTS

Bacterial isolates

A total of 71 nonrepetitive *P. aeruginosa* isolates were recovered, of which 37 (52.12%) were from males and 34 (47.88%) were from females. The age distribution of patients was as follows: <1 year old ($n = 13$, 18.31%), 1–15 years old ($n = 15$, 21.12%), 16–35 years old ($n = 7$, 9.86%), 36–55 years old ($n = 9$, 12.67%), and > 55 years old ($n = 27$, 38.03%). The origins of isolates were urine ($n = 31$, 43.66%), the respiratory tract ($n = 28$, 39.43%), blood ($n = 5$, 7.04%), eye swabs ($n = 3$, 4.22%), wound wabs ($n = 2$, 2.81%), ear swab ($n = 1$, 1.41%), and heart biopsy ($n = 1$, 1.41%).

Antimicrobial susceptibility profile

According to results from the antibiotic susceptibility test, 20 (28.17%) and 51 (71.83%) isolates were identified as CRPA and carbapenem-susceptible (CSPA), respectively. CRPA isolates were recovered from the respiratory tract ($n = 10$, 50%), urine ($n = 5$, 25%), blood ($n = 3$, 15%), and one wound and one ear swab ($n = 1$, 5% each). The resistance rates for β-lactams, including imipenem, meropenem, ceftazidime, and piperacillin/tazobactam were 26.76% ($n = 19$), 18.31% ($n = 13$), 22.53% ($n = 16$), and 18.31% ($n = 13$), respectively. The resistance rates for non-β-lactam antimicrobials, including ciprofloxacin, levofloxacin, gentamicin, amikacin, tobramycin, polymyxin B, and colistin were 25.35% ($n = 18$), 29.57% ($n = 21$), 21.12% ($n = 15$), 15.49% ($n = 11$), 19.72% ($n = 14$), 7.04% ($n = 5$), and 7.04% ($n = 5$), respectively. Polymyxin B and colistin showed the highest *in vitro* activity against the isolates, with a susceptibility rate 92.96% ($n = 66$) for each; while the highest resistance rate was seen against levofloxacin (29.57%, $n = 21$).

Except for polymyxin B and colistin, the degrees of resistance for all antibiotics tested were significantly higher in the CRPA group than the CSPA group [Table 2]. Although a significant percentage of both CSPA and CRPA isolates showed susceptibility to polymyxin B (90.2% vs. 100%) and colistin (92.15% vs. 95%), CSPA isolates were significantly more susceptible to the remaining antibiotics tested. Based on the criteria mentioned earlier, 11.26% ($n = 8$) and 15.49% ($n = 11$) were identified as the MDR-PA and XDR-PA phenotype,

respectively. The percentages of MDR-PA and XDR-PA among the CRPA were 35% and 50%, respectively; while the prevalence of MDR-PA and XDR-PA was low in the CSPA group (1.96% each).

Metallo-beta-lactamase activity and related genes

Of the 20 CRPA isolates, 4 (20%) were MBL-positive with the CDDST [Figure 1], and all showed multi-resistance patterns. Overall, 19 (95%) of CRPA isolates were found to carry MBL-encoding determinants [Figure 2]. The presence of MBL-encoding genes in CRPA isolates was not correlated with patient characteristics, including clinical specimen type, hospital ward, age, or gender ($P > 0.06$) [Table 3]. MBL genes detected were bla_{IMP} ($n = 18$, 90%), bla_{VIM} ($n = 8$, 40%), and bla_{NDM} ($n = 1$, 5%). The bla_{IMP} gene co-existed with bla_{VIM} in eight (40%) isolates. In addition, bla_{IMP} co-existed with the bla_{NDM} gene in only one (5%) CRPA isolate. Sequencing data

Table 2: Comparison of susceptibility of carbapenem-resistant *Pseudomonas aeruginosa* and carbapenem-susceptible *Pseudomonas aeruginosa* isolates to antibiotics tested

Antimicrobial agent	Number (%) of resistant isolates		P	Number (%) of susceptible isolates		P
	CRPA (n=20)	CSPA (n=51)		CRPA (n=20)	CSPA (n=51)	
CAZ	12 (60)	4 (7.84)	0.0001	8 (40)	46 (90.2)	0.0001
PTZ	10 (50)	3 (5.88)	0.001	8 (40)	46 (90.2)	0.0001
CIP	12 (60)	6 (11.76)	0.0001	7 (35)	44 (86.27)	0.0001
LEV	13 (65)	8 (15.68)	0.0001	5 (25)	43 (84.31)	<0.0001
GM	12 (60)	3 (5.88)	0.0001	8 (40)	47 (92.15)	0.0001
AK	8 (40)	3 (5.88)	0.007	12 (60)	48 (94.11)	0.007
TN	11 (55)	3 (5.88)	0.0001	9 (45)	48 (94.11)	0.0001
PB	0	5 (9.8)	0.9	20 (100)	46 (90.2)	0.9
CO	1 (5)	4 (7.84)	1	19 (95)	47 (92.15)	1

CAZ: Ceftazidime, PTZ: Piperacillin/tazobactam, CIP: Ciprofloxacin, LEV: Levofloxacin, GM: Gentamicin, AK: Amikacin, TN: Tobramicin, PB: Polymyxin B, CO: Colistin, CRPA: Carbapenem-resistant PA, CSPA: Carbapenem-susceptible PA, PA: *Pseudomonas aeruginosa*
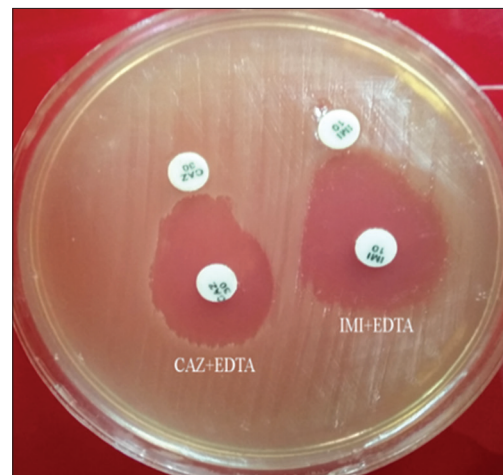

Figure 1: Phenotypic combined double-disk synergy test to detect metallo-beta-lactamase production in carbapenem-resistant P. aeruginosa isolates. Imipenem and imipenem + EDTA disks and ceftazidime and ceftazidime + EDTA disks were used to detect metallo-beta-lactamase -producers. A zone diameter difference between the disks alone and the disks plus EDTA of ≥8 mm was interpreted as metallo-beta-lactamase production

Table 3: Characteristics of carbapenem-resistant *Pseudomonas aeruginosa* isolated from hospitalized patients

Isolate	Patient characteristics				Resistance pattern	MDR	XDR	CDDST	MBL genes				
	Age (year)	Gender	Hospital ward	Isolation material					IMP	VIM	NDM	SIM	SPM
PA2	26	Female	Emergency room	Blood	IMI, MEM, CAZ, PTZ	+	-	-	+	+	-	-	-
PA3	78	Female	Internal medicine	Respiratory tract	IMI, CAZ, PTZ, LEV	-	+	+	-	+	-	-	-
PA6	83	Female	ICU	Respiratory tract	IMI, GM, CAZ, PTZ, CIP, LEV	-	+	-	+	+	-	-	-
PA7	71	Female	ICU	Respiratory tract	IMI, MEM, GM, AK, TN, PTZ, CIP, LEV	-	+	-	+	+	-	-	-
PA9	63	Male	Internal medicine	Respiratory tract	IMI, MEM, GM, TN, CAZ, CIP, LEV	-	+	-	+	-	+	-	-
PA11	79	Male	Outpataint	Urine	IMI, MEM, GM, TN, CIP, LEV	+	-	-	+	-	-	-	-
PA13	79	Male	Emergency room	Respiratory tract	IMI, MEM, GM, AK, TN, CIP	+	-	-	+	-	-	-	-
PA15	20	Female	Internal medicine	Respiratory tract	IMI, MEM, GM, AK, TN, CAZ, CIP, LEV	-	+	+	+	-	-	-	-
PA20	9	Male	Infectious diseases	Ear swab	IMI	-	-	-	+	-	-	-	-
PA21	52	Female	Outpataint	Urine	IMI	-	-	-	+	+	-	-	-
PA22	25	Male	Outpataint	Urine	IMI, GM, AK, CIP, LEV	+	-	-	+	-	-	-	-
PA25	68	Male	ICU	Respiratory tract	IMI, MEM, GM, CIP, LEV	+	-	+	+	+	-	-	-
PA31	1	Female	Outpataint	Urine	MEM	-	-	-	+	-	-	-	-
PA33	68	Male	Infectious diseases	Respiratory tract	IMI, MEM, GM, AK, TN, CAZ, PTZ, CIP, LEV	-	+	-	+	-	-	-	-
PA44	7	Female	Emergency room	Wound swab	IMI, MEM, GM, AK, TN, CAZ, PTZ, CIP	-	+	+	+	+	-	-	-
PA45	81	Female	Outpataint	Urine	IMI, CIP, LEV, CO	+	-	-	+	-	-	-	-
PA48	1	Female	PICU	Blood	IMI, MEM, CAZ, PTZ	+	-	-	+	+	-	-	-
PA52	46	Male	Nephrology	Blood	IMI, MEM, GM, AK, TN, CAZ, PTZ, CIP, LEV	-	+	-	-	-	-	-	-
PA55	1	Female	ICU	Respiratory tract	IMI, MEM, CAZ, PTZ, LEV	-	+	-	+	-	-	-	-
PA71	72	Female	Emergency room	Respiratory tract	IMI, GM, AK, TN, CAZ, PTZ, CIP, LEV	-	+	-	+	-	-	-	-

IMI: Imipenem, MEM: Meropenem, CAZ: Ceftazidime, PTZ: Piperacillin/tazobactam, CIP: Ciprofloxacin, LEV: Levofloxacin, GM: Gentamicin, AK: Amikacin, TN: Tobramicin, CO: Colistin, PA: *Pseudomonas aeruginosa*, MDR: Multidrug-resistant, XDR: Extensively drug-resistant, CDDST: Combined double-disk synergy test, MBL: Metallo-β-lactamase, ICU: Intensive Care Unit, PICU: Pediatric ICU, +: Yes, -: No
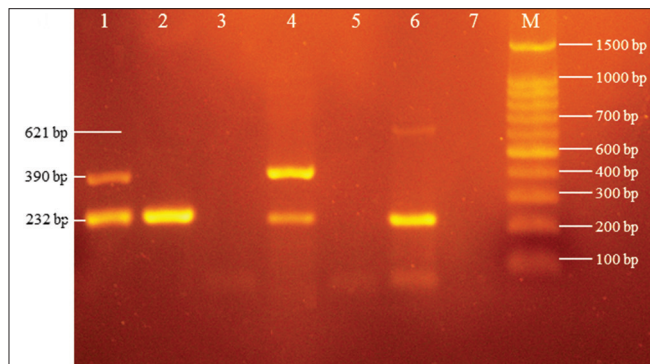

Figure 2: Agarose gel electrophoresis of multiplex PCR products. Lanes 1 and 4, isolates with blaIMP (232 bp) and *bla*~VIM~ (390 bp) genes; Lane 2, isolate with the *bla*~IMP~ (232 bp) gene; Lane 6, isolates with *bla*~IMP~ (232 bp) and *bla*~NDM~ (621 bp) genes; Lanes 3 and 5, isolates without any genes studied; Lane 7, negative control (distilled water); M, 100 bp DNA ladder

confirmed the presence of the bla_{IMP-1}, bla_{VIM-2}, and bla_{NDM-1} variants in isolates. No amplicon was obtained with primers targeting bla_{SPM}, and bla_{SIM}. All 4 isolates with MBL activity in the CDDST were positive for MBL genes, of which 1 (25%), 1 (25%), and 2 (50%) isolates carried the bla_{IMP}, bla_{VIM}, and

both genes, respectively. Furthermore, 15 (75%) of the CRPA which lacked MBL activity were positive for particular genes. Only one CRPA isolate was negative for MBL activity and any of the genes tested.

DISCUSSION

Antimicrobial resistance is an unpleasant event with a rising impact on patient safety, especially in Intensive Care Units (ICUs). Acutely, ill patients are prone to colonization and infection by antibiotic-resistant bacteria due to long-term hospitalization repeated exposure to antimicrobial agents and implementation of invasive therapeutic procedures. This serious array of risk factors leads to a vicious cycle of increased infection rates, the increased necessity for broad-spectrum antibiotics, reduced antimicrobial efficacy, and increased selection of drug resistance [15].

Carrying acquired MBL determinants enables bacteria to become resistant to broad-spectrum β-lactam antibiotics, including carbapenems, which are often the "last resort" agents for Gram-negative pathogens causing hospital-associated infections. Furthermore, MBL-producing pathogens usually develop to the multi-resistant phenotype, because they originate

nosocomially and acquired MBL-related genes typically cluster with other drug resistance determinants in the variable region of multi-resistance integrons [16]. These reasons explain why infections caused by MBL producers can pose a great challenge for antibiotic chemotherapy.

In this study, we described clinical carbapenem-resistant MDR/XDR *P. aeruginosa* isolates, which are capable of producing MBL type carbapenemase. Although the prevalence of CRPA in the present study was relatively low (28.17%), this rate of carbapenem resistance reflects a threat limiting treatment choices in our therapeutic centers. The ratio of CRPA differs by geographic region, kind of infection and specimen source, and selective pressure due to antibiotics [17]. In a recent study in Algeria, Meradji *et al*. [18] found that 18.75% of clinical *P. aeruginosa* isolates were resistant to carbapenems. In our country, there are different rates of resistance to carbapenems in *P. aeruginosa*. Mirsalehian *et al*. [19] reported a 100% rate of carbapenem-resistant and MDR-PA isolates from burn patients in Tehran, the capital of Iran. Furthermore, Farajzadeh Sheikh *et al*. [20] showed that among *P. aeruginosa* isolates from Ahvaz in Southwest Iran, 58.7% and 31.8% were resistant to imipenem and meropenem, respectively, and 44.4% showed multiple-drug resistance. However, lower prevalences of CRPA isolates were reported previously by Tarhani *et al*. [21], and Forozsh *et al*. [22]. In addition, we found a significantly higher multi-resistance rate in the CRPA group compared with the CSPA group, and the most effective antibiotics against both groups were polymyxin B and colistin. These findings indicate that polymyxins have increasingly become the last resort treatment for multi-resistant *P. aeruginosa* infections.

Various phenotypic methods, such as the MBL Etest, double-disk synergy test (DDST), and CDSST have been suggested for screening for MBL producing *P. aeruginosa*. Although both the DDST and CDDST are simple to perform and cheaper than the MBL Etest, they have shown incompatible results depending on the methodology used, β-lactam substrates, iMBLs, and the bacterial genus tested [13,20,23,24]. In the present study, 20% of all CRPA isolates were positive for MBL production on the phenotypic screening test, whereas 95% were positive for MBL-encoding genes. The reported low rates of MBL activity among the CRPA could be because detection of carbapenemase activity in clinical isolates is challenging work. In addition, discordant results for MBL detection between the phenotypic and genotypic methods were reported in Inacio *et al*.'s study [25], where 30% of *P. aeruginosa* were MBL producers with the Etest and DD methods using several iMBLs, whereas 87.5% were positive for the bla_{SPM-1}, and bla_{VIM-1} genes. In this manner, these MBL producers might act as silent reservoirs of such resistance determinants, with the ability to spread [26].

Overall, bla_{IMP} and bla_{VIM} are the most prevalent genes encoding MBLs detected in many regions, including Iran [27,28]. However, our study is in contrast to the findings obtained by Hakemi Vala *et al*. [29] and Bejestani *et al*. [30] in Tehran, Iran, who reported no bla_{VIM} producing-*P. aeruginosa* from clinical materials. There are few studies reporting the incidence of bla_{NDM} positive *P. aeruginosa* in our country. Most recently, Haghi *et al*. [31] found two clinical MDR-PA

harboring the bla_{NDM-1} in Zanjan, Iran. Although only one CRPA isolate was identified to carry this gene in the present study, it is of concern as bla_{NDM} producers can spread rapidly and this finding implies that numerous new bla_{NDM} cases will be found in the near future [32]. In addition, we found one non-MBL isolate negative for all genes tested. Therefore, the carbapenem resistance ability of the latter isolate may be mediated by other resistance mechanisms, such as loss of the *oprD* outer membrane protein-encoding gene, up-regulated efflux pumps, and production of other carbapenemases [33]. Further investigation is needed by our research group to address as many of these resistance mechanisms as possible.

Some correlation between the MBL-encoding genes of *P. aeruginosa* and patient characteristics has been demonstrated [34-36]. In a study in Brazil, Lucena *et al*. [34] found that ICU stay and urinary tract infections serve as significant risk factors for MBL-positive *P. aeruginosa* infections. Conversely, in two individual studies, Shirani *et al*. [37] and Zavascki *et al*. [38] found no significant correlations between MBL-positive *P. aeruginosa* and clinical characteristics of patients. Similarly, no specific characteristics of patients, including age, gender, hospitalization ward, and source of the isolate were associated with a higher frequency of MBL-positive *P. aeruginosa* infections in our study.

Herein, we demonstrated carbapenem-resistant MDR/XDR *P. aeruginosa* isolates with carbapenemase activity. IMP- and VIM-producing *P. aeruginosa* were the MBL-producing strains present at our therapeutic centers. The emergence of carbapenem resistance reflects a threat limiting treatment choices and suggests the need for ongoing epidemiological and antimicrobial susceptibility studies and longitudinal surveillance of antibiotic prescription. The continuous presence of such problematic organisms and their intra-hospital and inter-hospital spread is a matter of concern and should be taken into consideration by hospital infection control teams.

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Conflicts of interest

There are no conflicts of interest.

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