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Original Article

Emerge of NDM-1-Producing Multidrug-Resistant *Pseudomonas aeruginosa* and Co-harboring of Carbapenemase Genes in South of Iran

Ahmad FARAJZADEH SHEIKH [1,2], ****Mojtaba SHAHIN*** [3], ***Leili SHOKOOHIZADEH*** [4], ***Fahimeh GHANBARI*** [5], ***Hamid SOLGI*** [6], ***Fereshteh SHAHCHERAGHI*** [6]

1. *Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
2. *Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
3. *Department of Medical Laboratory Sciences, Faculty of Medical Sciences, Arak Branch, Islamic Azad University, Arak, Iran*
4. *Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamedan, Iran*
5. *Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
6. *Department of Bacteriology, Microbiology Research Center, Pasteur Institute of Iran, Tehran, Iran*

***Corresponding Author:** Email: shahin.mojtaba@yahoo.com

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Abstract

Background: New Delhi metallo-beta-lactamase-1 (NDM-1) is one of the most important emerging antibiotic resistance. Co-harboring three or four carbapenemases is rare and only a few reports exist in the literature. We described the characteristics of the large epidemic outbreaks and reports co-producing bla_{NDM-1} with the other carbapenemase genes in *P. aeruginosa* isolates.

Methods: This present cross-sectional research was conducted on 369 *P. aeruginosa* isolates obtained from burn and general hospitals within years 2013 to 2016. Beta-lactamase classes A, B and D genes were identified by PCR method. Modified hodge test (MHT), double-disk potentiation tests (DDPT) and double disk synergy test (DDST) were performed for detection carbapenemase and metallo beta-lactamase (MBL) production of bla_{NDM-1} positive *P. aeruginos* isolates.

Results: From 236 carbapenem-resistant *P. aeruginosa* (CRPA), 116 isolates have had MBL genes and twenty-nine isolates were found positive for bla_{NDM-1}. In CRPA isolates, bla_{IMP-1}, bla_{VIM-2} and bla_{OXA-10} were identified in 27.5%, 21.1% and 32.2% of isolates respectively, while co-producing bla_{NDM-1}, bla_{IMP-1}, bla_{OXA-10}, co-producing bla_{NDM-1}, bla_{VIM-2}, bla_{OXA-10} and co-producing bla_{IMP-1}, bla_{VIM-2} were determined in 11 (4.6%), 8 (3.4%) and 27 (11.4%) of isolates respectively.

Conclusion: The finding of this co-existence of multiple carbapenemase resistance genes is threating for public health. Dipicolinic acid is a superior MBL inhibitor in DDPT antique than EDTA in DDST method for the detection of MBL-bla_{NDM-1} producing *P. aeruginosa*

Keywords: *Pseudomonas aeruginosa*; bla_{NDM-1}; Modified hodge test (MHT); Double-disk potentiation tests (DDPT); Double disk synergy test (DDST)

Introduction

Pseudomonas aeruginosa is major agents of hospital-acquired pathogens (1). Carbapenemases indicate the most versatile family of beta-lactamase, with a wide spectrum inimitable by other beta-lactam hydrolyzing enzymes (2).

Carbapenems are the last-line treatment of multi-drug-resistant *P. aeruginosa* (MDRP) infections (1, 3). Because of the fact that carbapenems are a last resort treatment choice for infections caused by MDRP isolates, the presence of carbapenem-resistant strains is becoming a main public health challenge (2, 3). Among plasmid-mediated, extended-spectrum beta-lactamases (ESBLs) are commonly known to hydrolyze cephalosporins and metallo beta-lactamases (MBLs) can hydrolyze carbapenems.

Resistance to carbapenems can be related to producing carbapenemase enzymes such as serine carbapenemases (containing KPC and GES enzymes) and MBLs (metallo-beta-lactamases) such as imipenemase (IMP), Verona integrin-encoded metallo-β-lactamase (VIM) and New Delhi metallo-β-lactamase (NDM), enzymes and oxacillinases (such as OXA enzymes) (2, 4, 5).

MBLs such as bla_{VIM} and bla_{IMP} are the most clinically important classes of beta-lactamases; but the lately discovered transmissible New Delhi metallo beta-lactamase-1 (NDM-1) is becoming the most menacing in carbapenemase genes (2, 6). In addition, most bla_{NDM-1} strains are resistant to a wide-ranging of other antibiotic groups and transport numerous additional resistance genes for example to aminoglycosides, sulfonamides, macrolides and fluoroquinolones (7).

The detection of this co-harboring of multiple carbapenem resistance genes (Simultaneous attendance of both MBL and non-MBL genes) in clinical isolates from supremacy of carbapenems are considered as the last line resort of option for most of the dangerous infections caused by *P. aeruginosa*, but due to the prevalence of carbapenem-resistant *P. aeruginosa* (CRPA) isolates these lifesaving antibiotics were compromised in treating the patients with serious sickness (8).

The aims study were to identify the carbapenemase classes A, B and D and ESBL determinants among CRPA isolates in burn and non-burn patients. Moreover, identification of bla_{NDM-1} by three phenotypic methods (include DDPT, DDST and MHT) and comparing with PCR method was evaluated.

Materials and Methods

Bacterial isolation and identification

During the period from Oct 2013 to Jul 2016, 369 non-duplicate isolates were collected in burns (102 isolates from burn wounds) and general hospitals (267 from various hospital wards). These isolates were collected from teaching hospitals' microbiology laboratories in Ahvaz, Isfahan and Tehran cities from Iran. The isolation and identification of *P. aeruginosa* were done by the conventional methods and proved by PCR amplification with specific primers for *P. aeruginosa* gyrB gene with product size 221bp (9).

Antimicrobial susceptibility testing

The antibiotic susceptibility of all the isolates was tested by employing the Kirby-Bauer's technique as suggested by the CLSI (10). The eleven antibiotic disks used include: imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), cefepime (30 µg), cefotaxime (30 µg), amikacin (30 µg), gentamicin (10 µg), piperacillin/tazobactam (100/10 µg), aztreonam (30 µg) (Mast Group Ltd, UK). Isolates with resistance against a minimum of three groups of antibacterial agents were considered as MDR (11). To detect ESBL phenotype combined disk method using disks of ceftazidime (30 mg) with (10 mg) and without clavulanic acid (Mast Group Ltd, UK) was applied to all positively screened isolates by modified hodge test (MHT) (11). A growth in the area diameter of ≥5 mm around ceftazidime disc with and without clavulanic acid was expected to be a positive result for ESBL production (12, 13). The MHT was performed for all isolates as recommended by CLSI (10). The E test (imipenem 0.002-32µg/mL) (Liofilchem, Roseto degli Abruzzi, Italy) was applied (*according to the manufacturer's* instructions) to all positively screened isolates by PCR test for bla_{NDM} gene, to determine minimum inhibitory concentrations (MICs).

Phenotypic detection of MBLs

The double-disk potentiation tests (DDPT) and double disk synergy test (DDST) was performed for all bla_{NDM-1} positive (14, 15) for phenotypic detection of bla_{NDM-1} producing isolates. *The bacterial suspension with turbidity equivalent to 0.5 McFarland standard was prepared and cultured on MH agar.* Two *imipenem and imipenem*-EDTA disks and meropenem+Dipicolinic acid (Liofilchem, Roseto degli Abruzzi, Italy) were placed on the surface of the agar at a distance of 4 cm from each other. After 18-24 h of incubation at 35-37 °C, the inhibition zone of imipenem disks with imipenem alone and disks with imipenem plus 750 µg of EDTA were measured. An increase of 7 mm or more in the zone diameter for imipenem-EDTA disk in comparison with imipenem disk alone was considered as a MBLs producing isolate. Moreover, DDPT was interpreted as positive even if a small potentiation inhibition zone was present (14, 15).

PCR amplification of resistance genes

DNA of strains was extracted by the DNA extraction set (Sinaclon, Iran) based on *the* guidelines *of the manufacturer*. The specific primers were used for different types of carbapenemase (bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{GES}, bla_{SPM} and bla_{OXA-10}). In this study, pentaplex PCR was used for the rapid detection of MBL genes in CRPA isolates. The pentaplex PCR was optimized successfully to identify the MBL genes. Stepwise optimization of annealing temperature, primer concentration, $MgCl_2$, dNTP and Taq polymerase was performed. The pentaplex PCR gave the excellent results when 5 µL of 10X reaction buffer, 2 µL of 50 mM MgCl2, 1.5 µL of 2.5 mM dNTPs, 0.25 µL of each 10 pmol/µL primer, 0.5 µL Taq polymerase 5 U/µL, 37 µL distilled water and 55 °C annealing temperature were used (Fig. 1). The amplification reactions were carried out in a thermal cycler (Eppendorf AG, Germany), with an initial denaturation 4 min at 94 °C followed by 30 cycles of denaturation 60 sec at 94 °C, annealing 56 °C for bla_{OXA-10}, 59 °C for bla_{SPM} and 55 °C for pentaplex PCR and extension 60 sec at the temperature of 72 °C, with a single final extension of 7 min at 72 °C. The size of PCR products is determined by comparison with a DNA ladder (Sinaclon, Iran) on 1.5% agarose gels stained with ethidium bromide. Sequencing of the amplicons was performed by the Bioneer Company (Bioneer, Daejeon, South Korea). The nucleotide sequences were analyzed using blast in NCBI.

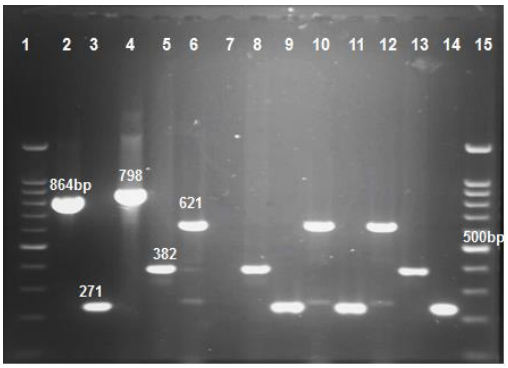

Fig. 1: Gel electrophoresis of multiplex PCR products following amplification with specific primers. Line 1 and 15 ladder, line2, 3, 4, 5 and 6 positive control bla_{KPC}, bla_{IMP}, bla_{GES}, bla_{VIM} and bla_{NDM} (864, 271, 798, 382 and 621 bp respectively), line 7 deionized water as control negative, line 8- 14 samples. All positive controls were provided by the Pasteur institute Iran

Ethics approval

This study was approved by the Medical Ethics Committee of Ahvaz Jundishapur University of Medical Sciences in Iran approved the study (permit number IR.AJUMS.REC.1395.227).

Results

Totally, of 369 confirmed *P. aeruginosa* isolates, 219 (59.3%) isolates were obtained from male and 150 (40.7%) isolates from female subjects. The majority 113 (30.6%) of isolates were obtained from punch/wound followed by 22.7% (84/369) from tracheal tube and 21.4% (79/369) isolates from urine samples. Seventy-four percent of all isolates were MDR (84% burn isolates and 67% from various hospital wards). Among all

isolates, 267 (72.3%) were carbapenem-resistant, meanwhile, the highest sensitivity was against to piperacillin-tazobactam 157 (42.5%). The full results of antibiotic resistance pattern of *P. aeruginosa* isolates shown in Table 1. MHT results showed that 236/369 (63.9%) isolates were positive as CRPA. Among CRPA isolates, high-level resistance to imipenem, meropenem and cefotaxime was observed. The comparison of antibiotic resistance of the CRPA in burn and non-burns isolates are shown in Table 2. Of 236 CRPA, 116 isolates (21 burn isolates and 95 isolates from various hospital wards) were MBL producing isolates, moreover, 105 (90.5%) were MDR isolates. In particular, this collection was included non-duplicate characterized bla_{VIM}, bla_{IMP} and bla_{NDM-1}.

Table 1: Antimicrobial susceptibility results of the all *Pseudomonas aeruginosa* isolates

Antimicrobial agent	***The number of P. aeruginosa***	***Number of Sensitive (%)***	***Number of Intermediate (%)***	***Number of Resistant (%)***
Imipenem	369	118(32)	22(5.9)	229(62.1)
Meropenem	369	118(32)	12 (3.2)	239(64.8)
Ertapenem	369	92(25)	10 (2.7)	267(72.3)
Piperacillin-Tazobactam	369	140(38)	72(19.5)	157(42.5)
Cfepime	369	130(35.3)	23(6.2)	216(58.5)
Amikacin	369	169(45.8)	20(5.4)	180(48.8)
Ciprofloxacin	369	120(32.5)	20(5.4)	229(62.1)
Gentamicin	369	134(36.3)	0	235(63.7%)
Ceftazidime	369	150(40.7)	16(4.3)	203(55)
Cefotaxime	369	25(6.8)	65(17.6)	279(75.6)
Azteronam	369	114(30.9)	121(32.8)	134(36.3)

Table 2: Antimicrobial susceptibility results of the CRPA in burns and non-burns isolates

Antimicrobial agent	***The number of CRPA isolates***		***Sensitive (%)***		***Intermediate) %)***		***Resistant (%)***	
	Burn patients	*Non-burn patients*	*Burn patients*	*Non-burn patients*	*Burn patients*	*Non-burn patients*	*Burn patients*	*Non-burn patients*
Imipenem	78	158	5(6.4)	7(4.4)	4(5.2)	6(3.8)	69(88.4)	145(91.8)
Meropenem	78	158	5(6.4)	6(3.8)	3(3.8)	8(5.1)	70(89.8)	144(91.1)
Ertapenem	78	158	2(2.6)	16(10.1)	2(2.6)	8(5.1)	74(94.8)	134(84.8)
Piperacillin-tazobactam	78	158	1(1.3)	46(29.1)	6(7.7)	50(31.6)	71(91)	62(39.2)
Cefepime	78	158	1(1.3)	26(16.4)	7(8.9)	11(7)	70(89.8)	121(76.6)
Amikacin	78	158	3(3.8)	64(40.5)	3(3.8)	12(7.6)	72(92.4)	82(51.9)
Ciprofloxacin	78	158	2(2.6)	23(14.5)	3(3.8)	8(5.1)	73(93.6)	127(80.4)
Gentamicin	78	158	4(5.2)	31(19.6)	0	0	74(94.8)	127(80.4)
Ceftazidime	78	158	21(26.9)	23(14.5)	7(8.9)	4(2.5)	50(64.2)	131(83)
Cefotaxime	78	158	2(2.6)	1(0.6)	2(2.6)	20(12.7)	74(94.8)	137(86.7)
Azteronam	78	158	8(10.2)	39(24.7)	32(41.1)	54(34.2)	38(48.7)	65(41.1)
Total	236(63.9) CRPA isolates							

The presence of bla_{IMP} and bla_{VIM} gene were detected in 21.6% (51/116) and 28.8% (68 isolates) of MBL producing isolates, respectively. The full results of antibiotic resistance pattern of bla_{IMP} and bla_{VIM} positive isolates in burns and non-burns isolates showed in Tables 3 and 4.

Twenty-four isolates from Ahvaz, 4 isolates from Isfahan and one isolate from Tehran in the collection was found carrying bla_{NDM-1} and confirmed by sequencing. The prevalence of ESBLs in MBL isolates was 11.2% (13/116) that 3 of them were bla_{NDM-1} isolates. Nineteen of bla_{NDM-1} isolates were co- harboring of two genes (bla_{VIM-2}/bla_{OXA-10} and bla_{IMP-1}/bla_{OXA-10}). Moreover, two bla_{NDM-1} isolates were co-harboring of three genes (bla_{VIM-2}, bla_{IMP-1} and bla_{OXA-10}). Moreover, 86.2% (25) bla_{NDM-1} positive isolates contained bla_{oxa-10}, simultaneously. Furthermore, bla_{KPC}, bla_{GES} and bla_{SPM} genes not found in none of the bla_{NDM-1} positive isolates. Unexpectedly, the results of DDST and DDPT revealed that 15(51.8%) and 26 (89.7%) of bla_{NDM-1} positive isolates were MBL producing isolates, respectively.

Table 3: Antimicrobial susceptibility results of VIM positive in burns and non-burns isolates

Antimicrobial agents	*The number of CRPA carrying VIM gene No. (%)*		*Sensitive No. (%)*		*Intermediate No.(%)*		*Resistant No. (%)*	
	Burn patients	Non-burn patients	Burn patients (%)	Non-burn patients (%)	Burn patients (%)	Non-burn patients (%)	Burn patients (%)	Non-burn patients (%)
Imipenem	18(17.6)	33(12.4)	1(5.5)	0	3(16.7)	2(6.1)	14(77.7)	31(93.9)
Meropenem	18(17.6)	33(12.4)	1(5.5)	1(3)	0	0	17(94.5)	32(97)
Ertapenem	18(17.6)	33(12.4)	1(5.5)	2(6.1)	0	1(3)	17(94.5)	30(90.9)
Piperacillin-tazobactam	18(17.6)	33(12.4)	1(5.5)	16(48.5	2(11.1)	12(36.4)	15(83.4)	5(15.1)
Cefepime	18(17.6)	33(12.4)	0	9(27.3)	1(5.5)	1(3)	17(94.5)	23(69.7)
Amikacin	18(17.6)	33(12.4)	2(11.1)	25(75.8)	1(5.5)	3(9.1)	15(83.4)	5(15.1)
Colistin	18(17.6)	33(12.4)	18(100)	32(97)	0	0	0	1(3)
Ciprofloxacin	18(17.6)	33(12.4)	0	7(21.2)	1 (5.5%)	2(6.1)	17(94.5)	24(72.7)
Gentamicin	18(17.6)	33(12.4)	0	9(27.3)	0	0	18(100)	23(82.3)
Ceftazidime	18(17.6)	33(12.4)	1(5.5)	7(21.2)	1(5.5)	1(3)	16(89)	25(75.8)
Cefotaxime	18(17.6)	33(12.4)	1(5.5)	0	1(5.%)	5(15.1)	16(89)	28(84.9)
Azteronam	18(17.6)	33(12.4)	3(16.7)	11(33.3)	2(11.1)	13(39.4)	13(72.2)	9(27.3)
Total		51 VIM isolates						

Discussion

Previously, only producers of the MBLs bla_{VIM} and bla_{IMP} had been detected. bla_{NDM-1} producing strains are surely threatening: firstly, bla_{NDM-1} encoding plasmids co-carriage multiple resistance determinants, they are commonly accounted as MDR isolates. Secondly, bla_{NDM-1} positive isolates have a potential for extent through the transfer of the plasmid bla_{NDM} gene (16). As explained previously, there are rare published reports of bla_{NDM-1} co-existence of multiple carbapenem resistance genes.

Table 4: Antimicrobial susceptibility results of IMP positive in burns and non-burns isolates

Antimicrobial agents	*The number of CRPA carrying IMP gene No. (%)*		*Number of Sensitive No. (%)*		*Number of Inter-mediate No. (%)*		*Number of Resistant No. (%)*	
	Burn patients	Non-burn patients	Burn patients (%)	Non-burn patients (%)	Burn patients (%)	Non-burn patients (%)	Burn patients (%)	Non-burn patients (%)
Imipenem	21(20.6)	47(17.6)	0	1(2.1)	0	1(2.1)	21(100)	45(95.8)
Meropenem	21(20.6)	47(17.6)	0	1(2.1)	0	0	21(100)	46(97.9)
Ertapenem	21(20.6)	47(17.6)	0	2(4.2)	1(4.8)	0	20(95.2)	45(95.8)
Piperacillin-Tazobactam	21(20.6)	47(17.6)	1(4.8)	17(26.5)	2(9.5)	26 (41.2)	18(85.7)	4(32.3)
Cefepime	21(20.6)	47(17.6)	2(9.5)	9(19.2)	1(4.8)	1(2.1)	18(85.7)	37(78.7)
Amikacin	21(20.6)	47(17.6)	1(4.8)	23(48.95)	1(4.8)	1(2.1)	19(90.4)	23(48.95)
Colistin	21(20.6)	47(17.6)	21(100)	45 (95.8)	0	0	0	2(4.2)
Ciprofloxacin	21(20.6)	47(17.6)	2(9.5)	5(10.6)	1(4.8)	1(2.1)	18(85.7)	41(87.3)
Gentamicin	21(20.6)	47(17.6)	1(4.8)	6(12.7)	0	0	20(95.2)	41(87.3)
Ceftazidime	21(20.6)	47(17.6)	2(9.5)	4(8.5)	1(4.8)	2(4.2)	18(85.7)	41(87.3)
Cefotaxime	21(20.6)	47(17.6)	0	0	0	3(6.4)	21(100)	44(93.6)
Azteronam	21(20.6)	47(17.6)	4(19)	7(14.9)	5(23.8)	18 (38.3)	12(57.2)	22(46.8)
Total	68 IMP isolates							

Infections with *bla*$_{NDM-1}$ producing isolates in non-endemic regions such as Europe and North America are often linked to visit and be hospitalized in endemic regions such as Indian subcontinent (17). The first report of *bla*$_{NDM-1}$ positive in *P. aeruginosa* came from Serbia (18). *bla*$_{NDM-1}$ producing *P. aeruginosa* is extremely rare (19). To date there are no reports of co-harboring occurrence *bla*$_{NDM-1}$ in *P. aeruginosa* isolates in Iran.

Nevertheless, *P. aeruginosa* isolates producing three carbapenemase genes is rare and has been reported in Brazil *(bla*$_{SPM-1}$*, bla*$_{KPC-2}$ *and bla*$_{VIM-2}$*) (20)*, Denmark (*bla*$_{NDM-1}$, *bla*$_{VIM-2}$, *bla*$_{IMP-1}$) (8), Bangladesh (*bla*$_{NDM-1}$, *bla*$_{VIM-2}$, *bla*$_{IMP-1}$) (21) and Turkey (*bla*$_{VIM-1}$, *bla*$_{VIM-2}$, and *bla*$_{GES-5}$) (22). Although these cases are scarce and sporadic, information of its occurrence is vital because NDM-positive *P. aeruginosa* is an organism with potent colonization ability in the hospital for long periods (23). To best of our knowledge, we report the first report of *P. aeruginosa* isolates producing four carbapenemases *co-existence bla*$_{NDM-1}$, *bla*$_{VIM-2}$, *bla*$_{IMP-1}$ and *bla*$_{OXA-10}$ from Iran. The acquisition of MBL-carbapenemase *bla*$_{NDM-1}$, *bla*$_{VIM}$, *bla*$_{IMP}$ and *bla*$_{SPM}$ led to emergence of MDR or XDR *P. aeruginosa (16).*

In the present study, imipenem resistance in burn and non-burn patients was 83.2% and 57.5% respectively. Imipenem was the ninth and fourth effective drugs in burn and non-burn isolates respectively, while in other researches particularly on burned patients in Iran, it was the most effective antipseudomonal antibiotic (24) in 10.8% of 415 isolates In burn patients, ceftazidime (with 26.9% sensitivity) and ertapenem, gentamicin and cefotaxime (with 94.8% resistance) and in non-burn patients amikacin (with 40.5% sensitivity) and imipenem (with 91.8%) resistance were the most and least effective antipseudomonal antibiotics. Even though, amikacin is the most effective antibiotic for infection of CRPA isolates, and also is a good drug for the treatment of non-burn isolates in CRPA isolates, but interestingly, we found that amikacin was a poorly antibiotic for burn infections due to CRPA isolates, the rate of resistance to this antibiotic was 92.4% which is relatively high in burn isolate. Similar to current study, another study among burned patients, reported 97.5% of *P. aeruginosa* isolates were resistant to imipenem and 90% of isolates resistant to amikacin (25). In Isfahan, surveyed 106 *P. aeruginosa* was isolated and 62 (58.5%) of isolates were imipenem resistance also MBL detected in

26| (42%) of them (26). In the current study, 21.6% and 28.8% of MBL producing strains, carried *bla*$_{IMP}$ and *bla*$_{VIM,}$ respectively. This rate is slightly higher than the result reported in previous studies, which can be a serious concern that may be because of a general increase in the extent of attainment of MBL genes among *P. aeruginosa*. This genes are found to be located on the class I integron and can hence quickly transfer among *P. aeruginosa* strains (27, 28). Compared to present study, lower resistant to imipenem (n=26, 25.2%), which 19 (73.0%) of them produce MBL, 6 (31.5%) samples had *bla*$_{VIM}$ gene and 2 (10.5%) had *bla*$_{IMP}$ gene. Lower percentage of IMP expression (10.5%) than our study has been also reported (29). One general concept has been evidenced that the quick appearance and dissemination of carbapenemase-producing strains is mostly due to the acquisition of *bla*$_{NDM}$ and *bla*$_{VIM-2}$ (7, 28). Antimicrobial susceptibility results of VIM and IMP positive isolates in burns and non-burns isolates indicated that high resistance to antibiotics. The corporation of other resistance determinants along with *bla*$_{VIM}$ confers the phenotype to become resistant to most of the accessible antibiotics (28). Aminoglycosides resistance genes on the similar gene cassette along with *bla*$_{VIM-2}$, therefore making the phenotype resistance to gentamicin and amikacin as well (30). Recognition of MBL-producing isolates can be effective for correct treatment of patients especially in burned patients (2). The mortality rate of patients infected with MBL-producing *P. aeruginosa* was higher (51.2%) than mortality caused by non-MBL-producing strains (32.1%) (31). Aztreonam is not appreciably hydrolyzed by NDM enzymes. Aztreonam was more effective than the carbapenems (31), but our study showed that 62% of these isolates were resistant to aztreonam. This occurrence of *bla*$_{OXA-10}$ was inside the range by Golshani (64%), Mirsalehian (74%), but more than other areas; however, *bla*$_{OXA-10}$ is prevalent in *P. aeruginosa* (24, 32).

Several phenotypic methods to detect MBL production have been developed, comprising the MHT, DDST, DDPT and E-test (15, 33). The MHT is the only CLSI recommended carbapenemase-screening method detected the weak carbapenemase activity enzyme. However, PCR is specific for detection of *bla*$_{NDM}$. The reports have shown a poor sensitivity of DDST and MHT phenotypic technique for detection *bla*$_{NDM}$, furthermore, due to its high false negative results, evaluating the performance of the MBL are needed (15, 33, 34). In the present study, 51.8% and 89.7% *bla*$_{NDM-1}$ isolates were positive in DDST and DDPT methods. There is a need for a more thorough evaluation of *bla*$_{NDM}$.in *P. aeruginosa* (35,36).

Conclusion

These findings imply the importance of *bla*$_{NDM-1}$ screening in Iran, which are being reported as potential regions of *bla*$_{NDM-1}$ endemicity. *The emergence of an acutely drug-resistant strain carrying multiple carbapenemase genes* is threating global health. Dipicolinic acid is a superior MBL inhibitor in DDPT than EDTA in DDST method for the detection of MBL-*bla*$_{NDM-1}$ producing *P.aeruginosa*. More research is needed to detect the *bla*$_{NDM-1}$ source.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

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