

RESEARCH

Open Access



Molecular characterization of *NDM-1*-producing *Pseudomonas aeruginosa* isolates from hospitalized patients in Iran

Mojtaba Shahin^{1,2} and Ali Ahmadi^{1*}

Abstract

Background: The emergence of carbapenem-resistant *Pseudomonas aeruginosa* is one of the most important challenges in a healthcare setting. The aim of this study is double-locus sequence typing (DLST) typing of *bla*_{NDM-1} positive *P. aeruginosa* isolates.

Methods: Twenty-nine *bla*_{NDM-1} positive isolates were collected during three years of study from different cities in Iran. Modified hodge test (MHT), double-disk synergy test (DDST) and double-disk potentiation test (DDPT) was performed for detection of carbapenemase and metallo-beta-lactamase (MBL) producing *bla*_{NDM-1} positive *P. aeruginosa* isolates. The antibiotic resistance genes were considered by PCR method. Clonal relationship of *bla*_{NDM-1} positive was also characterized using DLST method.

Results: Antibiotic susceptibility pattern showed that all isolates were resistant to imipenem and ertapenem. DDST and DDPT revealed that 15/29 (51.8%) and 26 (89.7%) of *bla*_{NDM-1} positive isolates were MBL producing isolates, respectively. The presence of *bla*_{OXA-10}, *bla*_{VIM-2}, *bla*_{IMP-1} and *bla*_{SPM} genes were detected in 86.2%, 41.4%, 34.5% and 3.5% isolates, respectively. DLST typing results revealed the main cluster were DLST 25-11 with 13 infected or colonized patients.

Conclusions: The presence of *bla*_{NDM-1} gene with other MBLs encoding genes in *P. aeruginosa* is a potential challenge in the treatment of microorganism infections. DLST showed partial diversity among 29 *bla*_{NDM-1} positive isolates.

Keywords: *Pseudomonas aeruginosa*, *bla*_{NDM-1}, DLST, MHT, MBL

Background

Pseudomonas aeruginosa is one of the most important hospital-acquired pathogens that causes miscellaneous opportunistic infections [1]. The emergence of multidrug-resistant (MDR: was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories) and extremely drug resistant (XDR: was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories) *P.*

aeruginosa isolates has been considered as a major concern for the treatment of infections caused by these isolates [2]. Carbapenemases are a wide spectrum group of beta-lactamase which hydrolyzes carbapenems to other b-lactams including monobactams, penicillins, and cephalosporins. Although carbapenems are a commonly last resort treatment used for MDR *P. aeruginosa* infection, the emergence of carbapenem-resistant *P. aeruginosa* is becoming a main public health concern and is associated with high rates of mortality and morbidity among hospitalized patients [3, 4].

Resistance to carbapenems can be related to producing carbapenemase enzymes such as serine carbapenemases and the MBLs encoding genes such as IMP, VIM, and

*Correspondence: Aliahmadigorgani@gmail.com

¹ Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
Full list of author information is available at the end of the article



© The Author(s) 2021, corrected publication 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

NDM enzymes [5]. The MBLs encoding genes such as *bla*_{VIM} and *bla*_{IMP} are one of the most clinically important classes of beta-lactamases; but, discovered transmissible New Delhi metallo-beta-lactamase-1 (*NDM-1*) is becoming the most threatening carbapenemase, recently [6–8]. The *bla*_{NDM-1} producing strains are resistant to a wide-ranging of other antibiotic groups and transport numerous additional resistance genes such as genes encoding resistance to fluoroquinolones, aminoglycosides, sulfonamides, and macrolides. Furthermore, the *NDM-1* enzyme is surfacing, resulting in almost whole resistance to antibiotics [8–10].

Molecular typing of *P. aeruginosa* is important to understand the local epidemiology, but it remains a challenging issue. The epidemiology of *P. aeruginosa* has been analyzed by an array of different typing methods such as Pulsed-field gel electrophoresis (PFGE) and Multilocus sequence typing (MLST) that are costly, required specific technical abilities and time to consume [11]. The newly described double-locus sequence typing (DLST) methods based on the partial sequencing of two highly variable loci to typing *P. aeruginosa* isolates which allowed us to obtain an unambiguous and standardized definition of types [12]. DLST has remarkable discriminatory power, reproducibility and is able to recognize high-risk epidemic clones [12]. Although *bla*_{NDM-1} positive isolates are rare, knowledge of its occurrence is considered as a serious menace, however, this study is the first report of DLST typing of *bla*_{NDM-1} positive *P. aeruginosa* isolates obtained from different part of Iran.

Methods

Study design, sampling, and bacterial isolates

A cross-sectional study was conducted at three major teaching Hospitals (Ahvaz, Tehran, and Isfahan) in Iran during three-year period. In total, 369 non-duplicate *P. aeruginosa* isolates were collected from different clinical sources such as trachea (84/369), wound (51/369), urine (79/369), punch biopsy (62/369), blood (34/369), sputum (35/369) and other (24/369). These samples were obtained from patient hospitalized in intensive care (ICU) and neonatal ICU (174/369), internal (149/369), emergency (11/369), other (15/369) and 20 samples from outpatients referred to laboratory center [13].

A total of 29 non-duplicate *bla*_{NDM-1} positive *P. aeruginosa* were collected from different clinical samples. The identification of *P. aeruginosa* was done by the conventional microbiology tests and confirmed by PCR with specific primers for *gyrB* gene [14].

PCR amplification of resistance genes

PCR amplification was performed for detection of *bla*_{NDM-1}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{KPC}, *bla*_{GES}, *bla*_{SPM} and

*bla*_{OXA-10} using a set of specific primers on a thermal cycler (Eppendorf AG, Germany) as described previously [15–17]. Sequencing of the amplicons was performed by the Bioneer Company (Bioneer, Daejeon, South Korea) and the nucleotide sequences were analyzed using GenBank nucleotide database at <http://www.ncbi.nlm.nih.gov/blast/>.

Antimicrobial susceptibility testing

Antibiotic susceptibility of the *bla*_{NDM-1} positive isolates was determined by the Kirby–Bauer method as recommended by the CLSI. The 11 standard antibiotic disks used include: imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), gentamicin (10 µg), piperacillin/tazobactam (100/10 µg), amikacin (30 µg), ciprofloxacin (5 µg) and aztreonam (30 µg) (Mast Group Ltd, UK). The ESBL phenotype was identified using combined disk method by disks of ceftazidime (30 mg) with (10 mg) and without clavulanic acid (Mast Group Ltd, UK), applied to all *bla*_{NDM-1} positive isolates (15). Moreover, the minimum inhibitory concentrations (MICs) of imipenem (10 µg/ml) [≤ 2 mg/L (susceptible), 4 mg/L (intermediate), and ≥ 8 mg/L (resistant)] (Liofilchem, Roseto degli Abruzzi, Italy) were applied by gradient test strips to *bla*_{NDM-1} positive *P. aeruginosa* isolates [18].

Carbapenemase screening

The double-disk potentiation tests (DDPT) and double disk synergy test (DDST) was performed phenotypically for all *bla*_{NDM-1} positive described by Yong et al. [19].

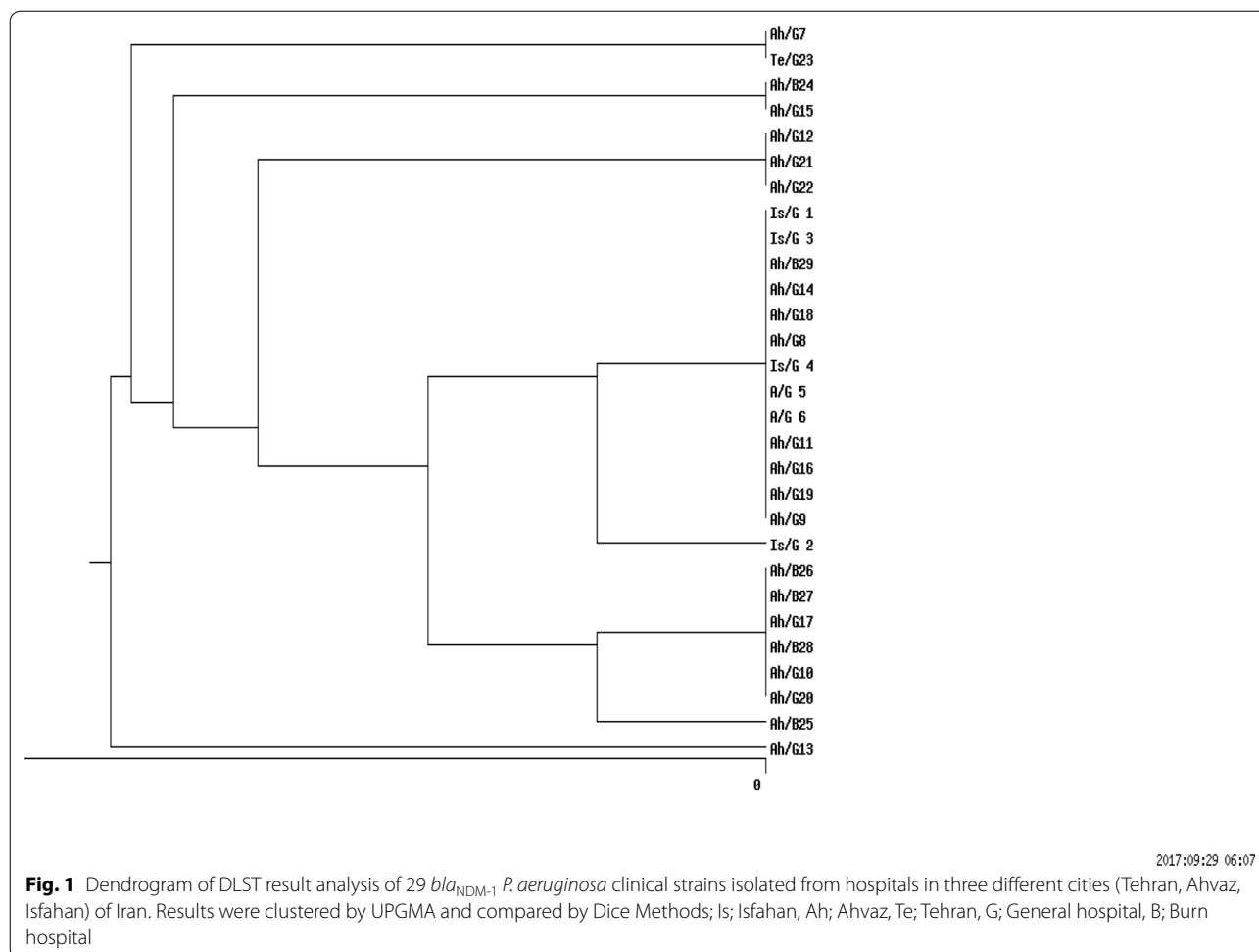
Double-locus sequence typing method

DLST typing was carried out using amplification of *ms172* and *ms217* loci using specific primers as previously described (Basset and Blanc, 2014) and according to DLST website (<http://www.dlst.org/>). PCR products were purified and were sequenced by Bioneer Corporation (Bioneer, Daejeon, South Korea).

In the cases of any results of allele assignment, the allele was considered as a null allele. The allele profiles were compared and clustered by the UPGMA and Dice methods (Fig. 1), using an online data analysis service (nslico.ehu.es).

Results

During the three years of study, twenty-nine of *bla*_{NDM-1} positive isolates were collected, of them, 6 (20.7%) and 23 (79.3%) isolates were from burn patients and non-burn patients, respectively. The male to the female proportion in *bla*_{NDM-1} isolates was 3.22 (n = 20:9). The most *bla*_{NDM-1} positive strains were isolated from wound/punch (n = 11; 37.9%) followed by urine (n = 11; 37.9%)



samples, whereas, the majority of the *bla*_{NDM-1} isolates were obtained from ICU ward ($n = 21$; 72.4%), followed by internal ward ($n = 6$; 20.7%) and burn ward ($n = 6$; 20.7%) (Table 1). Antibiotic susceptibility pattern showed that all isolates were resistance to imipenem and ertapenem, moreover, the resistance rate of meropenem was 96.5%. In contrast, the highest sensitivity was against to piperacillin/tazobactam and amikacin (41.4%). All *bla*_{NDM-1} positive isolates were defined as MDR. The full results of antibiotic resistance pattern of *bla*_{NDM-1} positive *P. aeruginosa* isolates showed in Table 2.

The MICs of imipenem against *bla*_{NDM-1} positive *P. aeruginosa* isolates are presented in Table 1. Overall, the results of MICs of imipenem showed 82.7% (24/29) isolates were high-level imipenem-resistant isolates (MIC ≥ 32). DDST and DDPT revealed that 15/29 (51.8%) and 26 (89.7%) of *bla*_{NDM-1} positive isolates were MBL producing isolates, respectively. In addition, the results of MHT showed that 27/29 (89.6%) of *bla*_{NDM-1} positive *P. aeruginosa* isolates were carbapenemase-producing isolates.

The distribution of carbapenemase genes and other antibiotic resistance genes among *bla*_{NDM-1} positive *P. aeruginosa* isolates are presented in Table 2. However, PCR analysis showed none of the *bla*_{NDM-1} positive *P. aeruginosa* isolates contained *bla*_{KPC} and *bla*_{GES} genes. The presence of *bla*_{VIM-2}, *bla*_{IMP-1} and *bla*_{SPM} genes were detected in 41.4%, 34.5% and 3.5% isolates, respectively. Among *bla*_{NDM-1} positive *P. aeruginosa* isolates, the *bla*_{OXA-10} beta-lactamase was the most frequently gene recognized in 86.2% (25/29).

The combination of *bla*_{VIM-2}, *bla*_{OXA-10} and *bla*_{IMP-1}/*bla*_{OXA-10} beta-lactamase was found in 8 (27.6%) and 10 (34.5%) isolates, respectively. Moreover, among *bla*_{NDM-1} positive *P. aeruginosa* isolates the co-harboring of three genes, *bla*_{OXA-10}, *bla*_{IMP-1} and *bla*_{VIM-2} was found in two isolates and only one isolate contained *bla*_{SPM} that in combination with *bla*_{VIM-2} and *bla*_{OXA-10}.

In the current study, the DLST method was tested for all *bla*_{NDM-1} isolates recovered over a period of four years from various hospital wards. DLST results revealed partial diversity among 29 *bla*_{NDM-1} positive isolates. Totally,

Table 1 The detailed results of *P. aeruginosa* with and without *bla*_{NDM-1}

Variable	<i>P. aeruginosa</i> (without <i>bla</i> _{NDM-1}) N=340 (%)	<i>P. aeruginosa</i> (with <i>bla</i> _{NDM-1}) N=29 (%)	p-value
Sex			
Male	199 (58.5)	20 (69)	0.2
Female	141 (41.5)	9 (31)	
Type of sample			
Trachea	80 (23.5)	4 (13.8)	0.2
Urine	68 (20)	11 (37.9)	197
Punch	60 (17.6)	2 (6.9)	0.1
Wound	42 (12.3)	9 (31)	0.005
Sputum	34 (10)	1 (3.5)	0.2
Blood	32 (9.4)	2 (6.9)	0.6
Other	24 (70.5)	–	–
Type of patients			
Burn	45 (13.2295)	6 (20.7)	
ICU	153 (45)	21 (72.4)	0.09
Internal	143 (42.1)	6 (20.7)	0.02
Emergency	11 (3.2)	–	–
Outpatients	18 (5.3)	2 (6.9)	0.7
Other	15 (4.4)	–	–

ICU Intensive care unit

8 different DLST profile (DL type) (four different common types and three single type) were detected (Table 2). The most common type including 13 isolates (45%) from different hospitals (in Ahvaz and Isfahan). A total of 29, 27 sequences were ms172 and ms217 whereas, 2 strains carried null alleles for these loci. The DLST profile 5–91 were detected in 3 (50%) burn isolates. The details of information about DL type of *P. aeruginosa* isolates are presented in Table 2.

Discussion

P. aeruginosa, one of the most common opportunistic pathogen associated with nosocomial infections, including pneumonia, urinary tract infections, and wound infections [1, 20]. Although carbapenems are often used as a therapeutic agent for treating infections caused by *P. aeruginosa*, the high emergence of carbapenem resistance significantly decreases their usefulness [21, 22]. The presence of *bla*_{NDM-1} producing isolates which may increase resistance to carbapenems is increasing among patients in healthcare systems [23]. In the current study, we described molecular characterization of *bla*_{NDM-1} producing of *Paeruginosa* isolates with phenotypic and genotypic methods.

The overall data show that the frequency of *bla*_{NDM-1} producing *P. aeruginosa* isolates was 7.8% (29/369). In addition, there are variable reports of *bla*_{NDM-1} from

different countries in Europe and Asian. The *bla*_{NDM-1} producing *P. aeruginosa* isolates has been also detected in Iran, recently. Shokri et al. from Isfahan (Center of Iran) in 2017 reported 6% of *P. aeruginosa* isolates were *bla*_{NDM-1} positive which is slightly lower than the result obtained in the present study [24]. In another study, Dogonchi et al. reported one isolate of *P. aeruginosa* harboring *bla*_{NDM-1} in the north of Iran [25]. Recently, Azimi et al. described a lower frequency rate for *bla*_{NDM-1} (7%) among carbapenem-resistant *P. aeruginosa* [26]. Moreover, the *bla*_{NDM-1} gene was also revealed by Riahi Rad et al. in Iran (21.4%) [27] and Takahashi et al. in Nepal (7%) [28].

With regard to the fact that our isolates were collected from hospitals of different cities in Iran and also regarding the results of previous studies, an increasing trend of *bla*_{NDM-1} producing *P. aeruginosa* strains can be observed in Iranian hospitals where it could be an endemic and serious concern in future. One of the important reason of possible increase of this phenomenon among Gram-negative isolates is inappropriate and excessive prescription and use of carbapenems in our hospitals, which leads to selective pressure.

According to our results, the most *bla*_{NDM-1} positive isolates (72.4%) were collected from the ICU ward that these findings are broadly consistent with the previous studies conducted in Iran [24, 25]. This finding suggests that the ICU ward is can be a risk factor and major source for the dissemination of resistant genes in the Iranian hospitals. Our results presented that *bla*_{NDM-1} positive isolates had highly resistant to all antibiotics commonly used in the clinic which is in agreement with to the results of other studies [24, 29, 30].

In spite of the fact that the *bla*_{NDM-1} gene demonstrating the sensitivity of bacteria to aztreonam, 62% of *bla*_{NDM-1} positive isolates were resistant to this agent that could be related to the presence of other beta-lactamase genes. Based on screening of other carbapenemase and metallo β -lactamase genes, *bla*_{OXA-10} was the most frequently detected beta-lactamase among *bla*_{NDM-1} positive strain and *bla*_{IMP-1} was second, which is in contrast to other reports where *bla*_{VIM} was significantly associated with *bla*_{NDM-1} [31, 32].

One of the important findings in this study was the emergence of the co-harboring of *bla*_{NDM-1} positive *P. aeruginosa* isolates with more than one carbapenemase gene and metallo β -lactamase determinants, simultaneously. Accordingly, we report the first isolate of *P. aeruginosa* producing four carbapenemases co-existence *bla*_{NDM-1}, *bla*_{VIM-2}, *bla*_{IMP-1} and *bla*_{OXA-10} from Iran. One of them that was obtained from urine sample was resistant to all antibiotics used except to TZP that were intermediate. Furthermore, we demonstrated the

Table 2 The detailed results of *bla*_{NDM-1} isolates

No.	Gender	City/hospital	Sample/ward	MB Ls genes Non-MB Ls Genes	Phenotypic tests			Antibiogram Pattern											DLST type
					Hodge test	MIC IPM	ESBL	IMP	MEM	ETP	TZP	CEP	AN	CI P	GEN	CAZ	CTX	AZT	
1	M	I/general	Wound/ICU	NDM-1, IMP-1, OXA-10	-	≥ 32	-	R	R	R	S	S	S	R	R	R	R	S	25-11
3	M	I/general	Urine/ICU	NDM-1, OXA-10	+	≥ 32	-	R	R	R	R	S	S	R	R	R	R	R	25-11
4	M	I/general	Urine/ICU	NDM-1, IMP-1, OXA-10	+	≥ 32	+	R	R	R	I	R	R	R	R	R	R	R	25-11
5	F	A/general	Urine/ICU	NDM-1, OXA-10	+	≥ 32	-	R	R	R	I	R	R	R	R	R	R	S	25-11
6	F	A/general	Urine/Internal	NDM-1, VIM-2, OXA-10	+	≥ 32	-	R	I	R	S	S	S	S	S	S	I	R	25-11
8	F	A/general	Blood/ICU	NDM-1, VIM-2, IMP-1, OXA-10	+	≥ 32	-	R	R	R	R	R	S	S	I	R	R	R	25-11
9	M	A/general	Urine/internal	NDM-1, OXA-10	+	≥ 32	-	R	R	R	R	R	S	I	R	R	R	R	25-11
11	M	A/general	Wound/internal	NDM-1, VIM-	+	8	-	R	R	R	S	R	S	R	R	R	R	S	25-11

Table 2 (continued)

				2, IM P-1															
14	M	A/general	Wound/ICU	ND M-1, VI M-2, OX A-10	+	≥ 32	-	R	R	R	S	R	S	R	R	R	R	S	25-11
29	F	A/burn	Wound/ICU	ND M-1, VI M-2, OX A-10	+	≥ 32	-	R	R	R	R	R	R	R	R	I	R	R	25-11
18	M	A/general	Urine/internal	ND M-1, OX A-10	+	≥ 32	-	R	R	R	I	R	S	R	R	R	I	R	25-11
19	M	A/general	Urine/ICU	ND M-1, IM P-1, OX A-10	+	≥ 32	-	R	R	R	I	R	I	R	R	R	S	R	25-11
16	M	A/general	Urine/NI CU	ND M-1, IM P-1, OX A-10	+	≥ 32	-	R	R	R	I	S	I	S	R	I	S	R	25-11
23	M	T/genera	Urine/ICU	ND M-1, OX A-10	-	2	+	R	R	R	R	R	R	R	R	S	R	I	32-39
7	M	A/general	Sputum/internal	ND M-1, IM P-1, OX A-10	+	≥ 32	-	R	R	R	I	R	R	R	R	R	R	R	32-39
25	M	A/burn	Punch/ICU	ND M-1, OX A-10	+	16	-	R	R	R	R	R	R	R	R	S	R	R	5-6
2	F	I/genera	Urine/ICU	ND M-1, IM P-1, OX	+	16	-	R	R	R	I	S	S	R	R	R	R	R	5-11

Table 2 (continued)

10	F	A/general	Urine/inte rnal	ND M- 1, VI M- 2, IM P-1, OX A- 10	+	≥ 32	-	R	R	R	I	R	R	R	R	R	R	5- 91	
17	M	A/general	Blood/OP	ND M- 1, IM P-1, OX A- 10	+	≥ 32	-	R	R	R	I	R	S	R	R	R	S	R	5- 91
26	M	A/burn	Punch/IC U	ND M- 1, OX A- 10	+	16	-	R	R	R	R	R	R	R	R	S	R	I	5- 91
27	M	A/burn	Wound/I CU	ND M- 1, OX A- 10	+	≥ 32	-	R	R	R	R	R	R	R	R	S	R	R	5- 91
28	F	A/burn	Wound/I CU	ND M- 1, VI M- 2, OX A- 10	+	≥ 32	-	R	R	R	I	R	R	R	R	S	R	I	5- 91
20	M	A/general	Trachea/I CU	ND M- 1, IM P-1, OX A- 10	+	≥ 32	-	R	R	R	S	R	S	R	R	I	R	R	5- 91
12	M	A/general	Trachea/I CU	ND M- 1, VI M- 2, OX A- 10	+	≥ 32	-	R	R	R	S	R	S	R	R	R	R	S	20- 68
21	M	A/general	Trachea/I CU	ND M- 1, IM P-1	+	≥ 32	+	R	R	R	R	R	R	R	R	R	R	R	20- 68
22	M	A/general	Trachea/I CU	ND M- 1, OX	+	≥ 32	-	R	R	R	R	R	S	R	S	R	R	I	20- 68

Table 2 (continued)

13	F	A/general	Wound/ICU	NDM-1, VIM-2	+	≥ 32	-	R	R	R	S	R	S	R	S	R	R	I	9-115	
15	M	A/general	Wound/OP	NDM-1, IMP-1	+	≥ 32	-	R	R	R	R	R	S	R	R	R	I	S	Nul I	
24	F	A/burn	Wound/ICU	NDM-1, VIM-2, OXA-10	+	≥ 32	-	R	R	R	R	R	R	R	R	R	I	R	R	Nul I

Imipenem (IPM), meropenem (MEM), ertapenem (ETP), piperacillin-tazobactam (TZP), cefepime (CFP), amikacin (AN), ciprofloxacin (CIP), gentamicin (GEN), ceftazidime (CAZ), cefexime (CTX), azteronam (AZT). A, Ahvaz; I, Isfahan; T, Tehran; OP, Outpatient; ICU

co-harboring of *bla*_{NDM-1} with metallo-β-lactamases genes such as *bla*_{OXA-10}, *bla*_{IMP-1} and *bla*_{VIM-2} in *P. aeruginosa*. The coexistence of carbapenemases encoding genes with *bla*_{NDM-1} positive *P. aeruginosa* isolates has been reported in several Asian and European countries including in India (*bla*_{NDM-1} + *bla*_{IMP} + *bla*_{VIM} + *bla*_{SPM}), Denmark (*bla*_{NDM-1} + *bla*_{VIM-5} + *bla*_{VIM-6}) [33], Bangladesh (*bla*_{NDM-1}, *bla*_{VIM-1}, *bla*_{VIM-2}, *bla*_{IMP-1}) [34] and Turkey (*bla*_{VIM-1} + *bla*_{VIM-2} + *bla*_{GES-5}) [35].

The previous studies revealed that the acquisition of MBL determinants such as *bla*_{NDM-1}, *bla*_{VIM}, *bla*_{IMP} and *bla*_{SPM} led to the emergence of MDR or XDR *P. aeruginosa* [36, 37].

To the extent of our knowledge, this study is the first to report of Molecular typing of *bla*_{NDM-1} positive isolates in *P. aeruginosa* by DLST method.

Among various genotyping technique, DLST as a reliable genotyping method, provide a new, rapid, typability, stability and low-cost of epidemiological surveillance of *P. aeruginosa* isolates [11, 38]. Analysis of DLST types revealed that the majority (19/29) of the isolates belonged to DLST type 25-11 and 5-91.

In addition, 8 *bla*_{NDM-1} positive isolates were clustered into two DLST types and 3 singletons. Accordingly, *bla*_{NDM-1} positive isolates were relatively heterogeneous, however, the route of transmission is not clear. These results highlight the importance of investigating carbapenem-resistant *P. aeruginosa* isolates in health care settings in our region.

Conclusions

The occurrence of *bla*_{NDM-1} isolates in *P. aeruginosa* is a large challenge in the treatment and worrying for global health. DLST type 25-11 is a significant cluster because a large number of *bla*_{NDM-1} isolates showed this genotype and also DLST type 5-91 known as an alarming type in burn patients. This work suggests that the DLST as an appreciated method in typing of *bla*_{NDM-1} strains; this technique reducing considerably the time and the cost of the molecular analysis and providing a reliable phylogenetic study. This information can help to generate the proper strategies for accurate and specific use of this antibacterial which can help to control of *bla*_{NDM-1} isolates.

Abbreviations

ESBL: Extended-spectrum b-lactamases; CLSI: Clinical and Laboratory Standards Institute; CCs: Clonal complex; DLV: Double-locus variants; DDST: Double-disk synergy test; DDPT: Double-disk potentiation test; KTP: Kidney transplant patients; ST: Sequence types; SLV: Single-locus variants; SD: Standard deviation; UPEC: Uropathogenic *Escherichia coli*; UTI: Urinary tract infections; MLST: Multilocus sequence typing; VFs: Virulence factors.

Acknowledgements

Thanks to guidance and advice from "Clinical Research Development Unit of Baqiyatallah Hospital".

Authors' contributions

AA designed the study and reviewed the manuscript, and edited the final version. MSH contributed to design the study, collected the data, and drafted the manuscript. AA analyzed the data, reviewed the manuscript, and edited the final version. All authors read and approved the final manuscript

Funding

Self-funding.

Availability of data and materials

Not applicable.

Declarations**Ethics approval and consent to participate**

This study was also confirmed and permitted by the Ethics Committee of Clinical Research Development Unit of Baqiyatallah Hospital" IR.BMSU. REC.1398.241".

Consent for publication

Not applicable.

Competing interests

The authors report no conflicts of interest in this work.

Author details

¹Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran. ²Department of Medical Laboratory Sciences, Faculty of Medical Sciences, Arak Branch, Islamic Azad University, Arak, Iran.

Received: 23 January 2021 Accepted: 19 October 2021

Published online: 03 November 2021

References

- Floret N, Bertrand X, Thouverez M, et al. Nosocomial infections caused by *Pseudomonas aeruginosa*: exogenous or endogenous origin of this bacterium? *Pathol Biol (Paris)*. 2009;57:9–12.
- Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18:268–81.
- Gniadek TJ, Carroll KC, Simner PJ. Carbapenem-resistant non-glucose-fermenting gram-negative bacilli: the missing piece to the puzzle. *J Clin Microbiol*. 2016;54:1700–10.
- Nakamura I, Yamaguchi T, Tsukimori A, et al. New options of antibiotic combination therapy for multidrug-resistant *Pseudomonas aeruginosa*. *Eur J Clin Microbiol Infect Dis*. 2015;34:83–7.
- Jeannot K, Poirel L, Robert-Nicoud M, et al. IMP-29, a novel IMP-type metallo- β -lactamase in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 2012;56:2187–90.
- Jamal WY, Albert MJ, Rotimi VO. High prevalence of New Delhi metallo- β -lactamase-1 (NDM-1) producers among carbapenem-resistant Enterobacteriaceae in Kuwait. *PLoS ONE*. 2016;11:e0152638.
- Kashyap A, Gupta R, Sharma R, et al. New Delhi metallo beta lactamase: menace and its challenges. *J Mol Genet Med*. 2017;11:1747–0862.
- Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev*. 2007;20:440–58. table of contents.
- Jovčić B, Lepšanić Z, Begović J, et al. Two copies of blaNDM-1 gene are present in NDM-1 producing *Pseudomonas aeruginosa* isolates from Serbia. *Antonie Van Leeuwenhoek*. 2014;105:613–8.
- Muir A, Weinbren MJ. New Delhi metallo-beta-lactamase: a cautionary tale. *J Hosp Infect*. 2010;75:239–40.
- Basset P, Blanc DS. Fast and simple epidemiological typing of *Pseudomonas aeruginosa* using the double-locus sequence typing (DLST) method. *Eur J Clin Microbiol Infect Dis*. 2014;33:927–32.
- Cholley P, Stojanov M, Hocquet D, et al. Comparison of double-locus sequence typing (DLST) and multilocus sequence typing (MLST) for the investigation of *Pseudomonas aeruginosa* populations. *Diagn Microbiol Infect Dis*. 2015;82:274–7.
- Farajzadeh Sheikh A, Shahin M, Shokoohzadeh L, et al. Molecular epidemiology of colistin-resistant *Pseudomonas aeruginosa* producing NDM-1 from hospitalized patients in Iran. *Iran J Basic Med Sci*. 2019;22:38–42.
- Lavenir R, Jocktane D, Laurent F, et al. Improved reliability of *Pseudomonas aeruginosa* PCR detection by the use of the species-specific *ectX* gene target. *J Microbiol Methods*. 2007;70:20–9.
- Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis*. 2011;17:1791–8.
- Shacheraghi F, Shakibaie MR, Noveiri H. Molecular identification of ESBL Genes blaGES-blaVEB-blaCTX-M blaOXA-blaOXA-4, blaOXA-10 and blaPER-in *Pseudomonas aeruginosa* strains isolated from burn patients by PCR, RFLP and sequencing techniques. *Int J Biol Life Sci*. 2010;3:138–42.
- Golshani Z, Sharifzadeh A. Prevalence of blaOxa10 type beta-lactamase gene in carbapenemase producing *Pseudomonas aeruginosa* strains isolated from patients in Isfahan. 2013;6.
- Wayne P. CLSI. Performance standards for antimicrobial susceptibility testing; twenty-second. Informational supplement CLSI document M100–S33. Wayne: Clinical and Laboratory Standards Institute; 2019.
- Yong D, Lee K, Yum JH, et al. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol*. 2002;40:3798–801.
- Alavi Foumani A, Yaghubi Kalurazi T, Mohammadzadeh Rostami F, et al. Epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis patients in Iran: a systematic review and meta-analysis. *Infez Med*. 2020;28:314–21.
- Ellappan K, Narasimha H, Kumar S. Coexistence of multidrug resistance mechanisms and virulence genes in carbapenem-resistant *Pseudomonas aeruginosa* strains from a tertiary care hospital in South India. *J Glob Antimicrob Resist*. 2018;12:37–43.
- Faghri J, Nouri S, Jalalifar S, et al. Investigation of antimicrobial susceptibility, class I and II integrons among *Pseudomonas aeruginosa* isolates from hospitalized patients in Isfahan, Iran. *BMC Res Notes*. 2018;11:806.
- Khajuria A, Praharaj AK, Kumar M, et al. Emergence of NDM-1 in the clinical isolates of *Pseudomonas aeruginosa* in India. *J Clin Diagn Res*. 2013;7:1328–31.
- Shokri D, Rabbani Khorasani M, Fatemi SM, et al. Resistotyping, phenotyping and genotyping of New Delhi metallo- β -lactamase (NDM) among Gram-negative bacilli from Iranian patients. *J Med Microbiol*. 2017;66:402–11.
- Dogonchi AA, Ghaemi EA, Ardebili A, et al. Metallo- β -lactamase-mediated resistance among clinical carbapenem-resistant *Pseudomonas aeruginosa* isolates in northern Iran: a potential threat to clinical therapeutics. *Tzu-Chi Med J*. 2018;30:90.
- Rad ZR, Rad ZR, Goudarzi H, et al. Detection of New Delhi Metallo- β -lactamase-1 among *Pseudomonas aeruginosa* isolated from adult and pediatric patients in Iranian hospitals. *Gene Rep*. 2021;23:101152.
- Azimi L, Fallah F, Karimi A, et al. Survey of various carbapenem-resistant mechanisms of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolated from clinical samples in Iran. *Iran J Basic Med Sci*. 2020;23:1396–400.
- Takahashi T, Tada T, Shrestha S, et al. Molecular characterisation of carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates in Nepal. *J Glob Antimicrob Resist*. 2021;26:279–84.
- Mohanam L, Menon T. Coexistence of metallo-beta-lactamase-encoding genes in *Pseudomonas aeruginosa*. *Indian J Med Res*. 2017;146:46–52.
- Shaaban M, Al-Qahtani A, Al-Ahdal M, et al. Molecular characterization of resistance mechanisms in *Pseudomonas aeruginosa* isolates resistant to carbapenems. *J Infect Dev Ctries*. 2017;11:935–43.
- Paul D, Dhar D, Maurya AP, et al. Occurrence of co-existing bla VIM-2 and bla NDM-1 in clinical isolates of *Pseudomonas aeruginosa* from India. *Ann Clin Microbiol Antimicrob*. 2016;15:31.
- Rahman M, Prasad KN, Gupta S, et al. Prevalence and molecular characterization of new Delhi metallo-beta-lactamases in multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from India. *Microbial Drug Resist*. 2018;24:792–8.
- Wang M, Borris L, Aarestrup FM, et al. Identification of a *Pseudomonas aeruginosa* co-producing NDM-1, VIM-5 and VIM-6 metallo- β -lactamases in Denmark using whole-genome sequencing. *Int J Antimicrob Agents*. 2015;45:324–5.
- Farzana R, Shamsuzzaman S, Mamun KZ. Isolation and molecular characterization of New Delhi metallo-beta-lactamase-1 producing superbug in Bangladesh. *J Infect Dev Ctries*. 2013;7:161–8.
- Malkocoglu G, Aktas E, et al. VIM-1, VIM-2, and GES-5 carbapenemases among *Pseudomonas aeruginosa* isolates at a tertiary hospital in Istanbul. *Turkey Microb Drug Resist*. 2017;23:328–34.

36. Paul D, Dhar Chanda D, Maurya AP, et al. Co-carriage of blaKPC-2 and blaNDM-1 in clinical isolates of *Pseudomonas aeruginosa* associated with hospital infections from India. *PLoS ONE*. 2015;10:e0145823.
37. Flateau C, Janvier F, Delacour H, et al. Recurrent pyelonephritis due to NDM-1 metallo-beta-lactamase producing *Pseudomonas aeruginosa* in a patient returning from Serbia, France, 2012. *Eurosurveillance*. 2012;17:20311.
38. Pappa O, Beloukas A, Vantarakis A, et al. Molecular characterization and phylogenetic analysis of *Pseudomonas aeruginosa* isolates recovered from

Greek aquatic habitats implementing the double-locus sequence typing scheme. *Microb Ecol*. 2017;74:78–88.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

