

## Prevalence of Clinically Isolated Metallo-beta-lactamase-producing *Pseudomonas aeruginosa*, Coding Genes, and Possible Risk Factors in Iran

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### ABSTRACT

**Background & Objective:** The spread of carbapenem-resistant *Pseudomonas aeruginosa* is a global concern. Metallo-beta-lactamase (MBL) enzymes cause extensive drug resistance among Gram-negative bacteria. The current study aimed at determining the prevalence of MBL-producing *P. aeruginosa* in Iran.

**Data extraction:** A total of 43 studies were found out of which 36 were adopted. Data were collected from Google, Google Scholar, Science Direct, PubMed, Scopus, Embase, and Sciverse. The terms “*Pseudomonas aeruginosa*”, “metallo-beta-lactamase”, “prevalence”, “carbapenems”, and “Iran” were searched. Data from the isolates not producing MBLs were excluded from the study. Data were analyzed with Graph Pad Prism 6, meta-analysis section.

**Results:** According to the results of the current study, 36 surveys indicated that 55% of the clinically isolated *P. aeruginosa* in Iran were resistant to imipenem and meropenem, among which 37.72% were the MBL producers. Among genes encoding MBLs, *bla<sub>VIM</sub>* and *bla<sub>IMP</sub>* were predominant with the prevalence of 12.91%±11.01% and 12.50%±23.56%, respectively. No report of harboring *bla<sub>NDM1</sub>* and *bla<sub>SPM1</sub>* by *P. aeruginosa* was found, similar to most of the other countries in Asia. The prevalence of *bla<sub>VIM</sub>* and *bla<sub>IMP</sub>* from burn settings were 11.50%±3.5% and 24.65%±23%, respectively. Furthermore, the prevalence of these genes was not significantly different among burn and non-burn isolates (P=0.942 and P=0.597, respectively). Moreover, no relationship was observed between the MBL production and patients’ age range.

**Conclusion:** Approximately half of *P. aeruginosa* isolates were carbapenem-resistant in Iran, and approximately half were the MBL producers. The *bla<sub>VIM</sub>* and *bla<sub>IMP</sub>* were the predominant MBLs among *P. aeruginosa* strains, while other genes were not found in *P. aeruginosa*. Moreover, there was no significant difference between *bla<sub>VIM</sub>* and *bla<sub>IMP</sub>* among burn and non-burn isolates. Due to the multiple drug resistance conferred by MBLs, detection and control of their spread alongside proper therapeutic regimens in hospitals and community settings are essential to prevent infection acquisition.

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### Introduction

Carbapenem resistance among clinically isolated *Pseudomonas aeruginosa* (*P. aeruginosa*) is a great concern worldwide, as this class of antibiotics is among the last resorts to eradicate infections with

Gram-negative species (1-3). The prevalence of multidrug-resistant *P. aeruginosa* (MDRP) non-susceptible to quinolones and aminoglycosides in addition to beta-lactams is reported worldwide (4-6). *Pseudomonas aeruginosa* isolates acquire resistance to carbapenems via several mechanisms

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including overexpression of efflux systems, change or lack of outer membrane proteins (such as OprD porin), chromosomal AmpC beta-lactamase, and production of carbapenemases, overall named heteroresistance (7). The most important carbapenemases produced by *P. aeruginosa* are zinc-dependent metallo-beta-lactamases (MBLs) capable of hydrolyzing imipenem, meropenem, ertapenem, and cephalosporins, but not monobactams and aztreonam (8, 9). Other types of carbapenemases include class D enzymes such as OXA-23, OXA-27, OXA-48, and class A including clavulanic acid inhibitory enzymes (SME, NMC, IMI, and KPC) (10). MBLs are determined in Enterobacteriaceae and other Gram-negative non-fermenters (8). Phenotypic detection of MBLs include (1) Combined disk using imipenem and imipenem + EDTA (ethylenediaminetetraacetic acid)/dipicolinic acid; 2) The Hodge test in which *Escherichia coli* ATCC is lawn on Mueller-Hinton agar, and then, test strains are cultured horizontally, and 3) The carbaNP test as the most sensitive protocol. Carbapenem and vancomycin exposure were shown as risk factors for carbapenem-resistant *P. aeruginosa* in Brazil (11). There are various MBL genes among carbapenem-resistant *P. aeruginosa* including Verona integron-encoded MBL (*VIM*), Germany imipenemase (*GIM*), imipenemase (*IMP*), Sao Paulo MBL (*SPM*), New Delhi MBL (*NDM*), and Florence imipenemase (*FIM*). Each MBL gene is encoded by specific genetic elements including transposons, integrons, plasmids, or on the chromosome, carrying genes encoding determinants of resistance to several antibiotics in addition to carbapenems, causing the advent of MDR *P. aeruginosa*. Moreover, these genetic determinants are transferable to other Gram-negative species, extending the antimicrobial resistance rate and complicating the treatment of infected patients (12). Therefore, it is necessary to understand the epidemiology, molecular characteristics, and resistance mechanism of MPPA to control infection and prevent a possible global health crisis. These beta-lactamases are inhibited by the EDTA chelator. The most important MBL types for epidemiological spread and clinical relevance are *IMP*, *VIM*, *SPM*, and currently *NDM* (13, 14). The predominant MBLs are *VIM* and *IMP*

reportedly carried by the mobilizable elements such as integrons. The *VIM-2* integrons are detected in MDR strains (15). It is reported that *VIM*-type MBLs are predominant in some areas (16, 17). The *bla<sub>NDM1</sub>* was reported from *Klebsiella pneumonia* in Iran in 2013 (18).

### Objectives

The current study aimed at investigating the MBL production and MBL types among clinically isolated *P. aeruginosa* strains in Iran.

### Data extraction

The current meta-analysis review collected data from search engines such as Google, Google Scholar, Science Direct, PubMed, Scopus, Sciverse, etc. The terms “*Pseudomonas aeruginosa*”, “metallo-beta-lactamas”, “prevalence”, “carbapenems” and “Iran” were searched. A total of 43 studies were found out of which 36 were adopted. Studies on phenotypic results of carbapenem resistance were included. All studies on burn isolates were also included. Studies on other mechanisms of carbapenem resistance were excluded. Data from non-metallo-beta-lactamase-mediated carbapenem-resistant strains were also excluded from the study. The prevalence of phenotypic and molecular studies was transferred into the software and the results were analyzed. The bias among published studies were the risk factors, sample size, the outcome of infections, the genetic relationship between strains in hospital settings (if the infection is clonally spread), age, and the economic stats of patients. The current study inclusion criteria were the possible influence of these risk factors on the prevalence of MBL-producing *P. aeruginosa* alongside the genetic characteristics of the strains.

Data were analyzed using GraphPad Prism 6, meta-analysis section, and the standardized mean difference by the Cohen *d* method basically reached by the employment of difference score divided by standard deviation (SD) of the scores analyzed by the software itself.

### Results

The previous studies demonstrated that 55% of *P. aeruginosa* isolates were resistant to carbapenems. Of them, 37.72% were MBL-

positive. The mean prevalence of *bla<sub>VIM</sub>* was predominant, while only 1% of the strains were *bla<sub>IMP</sub>* producers and none were positive for *bla<sub>NDM1</sub>* and *bla<sub>SPMI</sub>* (Table1) (19-26).

Furthermore, no significant difference was observed among burn and non-burn isolates regarding the prevalence of MBL genes. There was no significant relationship between patients' age ranges and the presence of MBLs. Furthermore, the mortality rate due to infection with MBL-producing bacteria was not fully elucidated, according to the data from some studies (47.25±3795, N=2).

As already mentioned, the bias among the published studies were seldom the detection of risk factors, sample size differences, lack of data on the outcome of infections, the obscure genetic relationship of strains within hospital settings (if the infection is clonally spread), weak uncovering ages, and the economic status of the patients. Hence, the possible risk factors for the acquisition of MBL-producing *P. aeruginosa* strains were not potentially achieved.

**Table1.** Phenotypic and Genotypic Prevalence of MBL Types in Different Cities of Iran

MBL Type	Percentage (%)	City	Total Isolates	MR (%)	Resistance	Year	Reference
VIM	6.34	Tehran	126	-	Imipenem	2007	
	19.51	Ahvaz	100 <sup>B</sup>	-	Imipenem	2008	(27)
	17.30	Tabriz	104	82.6	Imipenem	2010	(19)
	12.36	Tehran	186 <sup>B</sup>	-	Imipenem	2010	(24)
	2.62	Tehran	610	-	Imipenem	2010	(22)
	13 <sup>a</sup>	Tehran	100 <sup>B</sup>	-	Imipenem	2010	(28)
	16.1	Tehran	483	-	Imipenem	2010	(21)
	2.1 <sup>a</sup>	Tehran	483	-	Imipenem	2012	(29)
	32.85	Zanjan	70	-	imipenem	2012	(29)
	5.17	Urmia	58	-	Imipenem	2013	(30)
	7.5 <sup>a</sup>	Shiraz	240 <sup>B</sup>	-	Imipenem	2013	(31)
	21.3, 24 <sup>a</sup>	Arak	108	-	Imipenem	2012	(32)
	0.46	Kermanshah	225	-	Imipenem, meropenem	2014	(33)
						2015	(34)
	IMP	5.76	Tabriz	104	-	Imipenem	2010
57.9		Tehran	100	8.3	Imipenem	2013	(26)
6.6		Tehran	483	-	Imipenem	2012	
3.3 <sup>b</sup>		Tehran	483	-	Imipenem	2012	
14.28		Zanjan	70	-	Imipenem	2013	(30)
3.41		Urmia	58	-	Imipenem		
7.5 <sup>b</sup>		Shiraz	240	-	Imipenem	2013	
1.3		Tehran	75	-	Imipenem	2012	
15.11		Kermanshah	225 <sup>B</sup>	-	Imipenem, meropenem	2012	(35)
					2015	(34)	

MBL, metallo-beta-lactamase; <sup>a</sup>VIM-2; <sup>b</sup>IMP-2; <sup>B</sup> isolates from burn injuries; MR, mortality rate

### Worldwide incidence of MBL-producing *P. aeruginosa*

#### Middle-East and North African countries

A study by Bahar from Turkey showed the presence of *VIM-5* beta-lactamase for the first time in *Klebsiella pneumoniae* (36). Iraz reported *bla<sub>GES-5</sub>* and a novel *bla<sub>VIM</sub>* variant, named *VIM-38* (37). Other studies from Saudi Arabia demonstrated the presence of *VIM-2* in a patients with HIV (38), and 41% (16/39) *bla<sub>VIM</sub>* among the extended-spectrum beta-lactamases (ESBL)-producing *P. aeruginosa* (39). Another study revealed that 20.57% (72/350) of the isolates produced MBL and all of them

carried *bla<sub>VIM-2</sub>* (40). In Egypt, the first report of *bla<sub>NDM-1</sub>* associated with *bla<sub>VIM-2</sub>* was published in 2014 (41). Another study from Egypt revealed that 39.34% of *P. aeruginosa* species isolated from patients with cancer were imipenem-resistant among which 27% were the MBL-positive strains. The *bla<sub>VIM-2</sub>*, *bla<sub>NDM-1</sub>*, and *bla<sub>IMP-1</sub>* were detected among 58.3%, 4.2%, and 2.1% of MBL-positive isolates (42). The *VIM-2* was reported from Tunisia and Algeria in North Africa (43-45). In a study in Tunisia, of 30 MBL-positive isolates, 17.5% were *bla<sub>VIM-2</sub>* positive (46).

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### Asia Pacific

In a study by Dong in China, among 59 carbapenem-resistant *P. aeruginosa* strains, 39 (66.1%) were positive for the MBL genotype; 35 (89.7%) and 4 (10.3%) of which carried *bla*<sub>IMP-1</sub> and *bla*<sub>VIM-2</sub>, respectively (47). Qu showed that among 24 MBL-producing strains, 10 were positive for the *bla*<sub>VIM-2</sub>, while 13 were positive for *bla*<sub>IMP-9</sub>, and 1 for *bla*<sub>IMP-1</sub> (48). Yu showed that 14/140 of *P. aeruginosa* species were positive for *bla*<sub>VIM-2</sub>; in addition 12 of which carried class 1 integrons (49). The *bla*<sub>IMP-9</sub> was first reported from China in 7 *P. aeruginosa* isolates in 2005 (50). In Southern China, only 1 of 61 imipenem-resistant isolates harbored *bla*<sub>IMP-9</sub> (51). Of 368 isolates from several cities in China, 25 were positive for the *bla*<sub>IMP-6</sub> and 3 positive for *bla*<sub>VIM-2</sub> with the predominance of ST244 and ST235 sequence types (52). The *bla*<sub>KPC-2</sub> was detected among 38 carbapenemase-producing *P. aeruginosa* isolates exhibiting ST463 (53). In Thailand, MBL was clearly positive in 24 (18.46%) and weakly positive in 12 (9.23%) isolates; *IMP-1*, *IMP-14*, and *VIM-2* were detected among both of these sets of isolates (54). In Japan, 11 of 23 carbapenem-resistant isolates carried *GES-5* (55). Another study showed that the prevalence of *bla*<sub>IMP</sub>/*(AAC-6)*-producing *P. aeruginosa* increased in Japan from 170/300 (56.7%) in 2011 to 230/300 (76.7%) in 2012 (56). In Korea, 20 (15.6%) and 11 (8.6%) imipenem non-susceptible isolates were positive for *bla*<sub>IMP-1</sub> and *bla*<sub>VIM-2</sub>, respectively (57).

### European countries

The *bla*<sub>GIM-1</sub> was first reported from Germany among 28% of the isolates in 2004 (58). In the study by Valenza in Germany, among 489 isolates, 11.7% of MBL-producing isolates were *bla*<sub>VIM</sub>-positive, but no other encoding gene was detected (59). Another study showed the outbreak of *bla*<sub>VIM-2</sub> among 11 specimens from urinary tract infection in Germany (60). There was a case report of NDM-1-producing *P. aeruginosa* in France (61). There was another report of *bla*<sub>NDM-1</sub> in Balkan region, Serbia (62) as well as a report of *IMP-29* in France (63). A novel MBL named *VIM-14* carried in a class 1 integron with a new organization was detected in Italy. The integron harbored the genes *aac7*, *bla*<sub>VIM</sub>.

*14*, *bla*<sub>OXA-20</sub>, and *aac4* (64). A novel *bla*<sub>IMP</sub>, *bla*<sub>IMP-58</sub>, was recently reported from Denmark (65).

### North America

The first report of MBL-producing *bla*<sub>VIM-2</sub> in the United States was in 2005 (66). The *bla*<sub>NDM</sub> was reported in some Enterobacteriaceae isolates in USA (67).

### Latin America and Africa

In a study in Brazil, MBLs included SPM-1 (55.6%), VIM-2 (30.6%), and IMP-1 (8.3%) enzymes (68). The *NDM-1* was detected in *K. pneumonia* in South Africa (69).

### Discussion

In the current review, the range of MBLs was from 16.68% in the study by Shahcheragi to 100% in the studies by Bahar and Saderi (21-23). The variation between phenotypic and genotypic detection of MBLs were not highlighted among previous studies from Iran, although it was reported by other studies (70). The studies showed that the presence of other resistance mechanisms may interfere in the MBL phenotypic detection, or EDTA can affect cell membrane (71). The *NDM1*, *SPM1*, and *GIM* genes are not present in Iran, and are reportedly detected in distinct geographic areas (5, 72, 73). Rojo Bezares demonstrated that 49.4% of carbapenem-resistant *P. aeruginosa* from Spain were MBL-positive, all were *bla*<sub>VIM2</sub>-positive, and 75% were integron-class1-positive (74). In the study by NagKumar, 18.85% of *P. aeruginosa* isolates were MBL-positive. In Colombia, 60% of carbapenem-resistant strains were VIM-positive, but all were *IMP*- and *NDM*-negative, and also class 1 and 2 were detected among them (75). The *GIM1*, *SPM1*, and *FIM1* were reported from Germany, Switzerland, Brazil, and Italy, respectively (58, 76-78). The *bla*<sub>VIM</sub> is the main MBL encoding gene among Middle-East and most of other Asian countries. The *bla*<sub>NDM-1</sub> emerged in some countries other than India, and thus, its spread is of great concern.

The current study determined no significant difference regarding the prevalence of *bla*<sub>IMP</sub> and *bla*<sub>VIM</sub> between the species isolated from burn and non-burn injuries.

Furthermore, it was not revealed if there was a relationship between the prevalence of MBLs and patients' age ranges. In addition, the mortality rate was not fully elucidated; however, results of 2 studies showed a mean rate of 47.25%. It was depicted that previous antibiotic use, catheterization, intravenous (IV) lines, >8 days hospital stay, mechanical ventilation, and endotracheal intubation were the risk factors for MBL-producing *P. aeruginosa*, but significant risk factors for MBL-positives species were graft application and surgical intervention (79). As already mentioned, the bias among published studies seldom detected risk factors, sample size differences, lack of the outcome of infections, the obscure genetic relationship of strains within hospital settings (if the infection was clonally spread), and weak uncovering ages and the economic status of the patients. For these reasons, the possible risk factors for the acquisition of MBL-producing *P. aeruginosa* infections were not potentially achieved.

### Conclusion

Approximately half of *P. aeruginosa* isolates were carbapenem-resistant in Iran, among which nearly half were MBL-positive. The *bla<sub>VIM</sub>* and *bla<sub>IMP</sub>* were the predominant MBLs in *P. aeruginosa* strains, and the emergence of other genes is a concern. Moreover, there was no significant difference between *bla<sub>VIM</sub>* and *bla<sub>IMP</sub>* prevalence among burn and non-burn injuries. Due to multiple drug resistance conferred by MBLs, detection and control of their spread alongside proper therapeutic regimens in hospital and community settings is essential.

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### Conflict of interest

The authors declared no conflict of interest.

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