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Prevalence of metallo- β -lactamase genes among *Pseudomonas aeruginosa* isolated from various clinical samples in China

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Abstract

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen which is associated with nosocomial infections and causes various diseases including urinary tract infection, pneumonia, soft-tissue infection and sepsis. The emergence of *P. aeruginosa*-acquired metallo- β -lactamase (MBL) is most worrisome and poses a serious threat during treatment and infection control. The objective of this study was to identify antibiotic susceptibility, phenotypic detection of MBL production and to determine the prevalence of MBL genes in carbapenem-resistant *P. aeruginosa* isolated from different clinical samples.

Methods: A total of 329 non-duplicate *P. aeruginosa* isolated from various clinical samples from two hospitals in China between September 2017 and March 2019 were included in this study. Phenotypic detection of MBL was performed by the combined detection method using imipenem and imipenem-ethylenediaminetetraacetic acid (EDTA) discs. MBL-encoding genes including bla_{VIM-1}, bla_{VIM-2}, bla_{IMP-1}, bla_{IMP-2}, bla_{SPM-1}, bla_{SIM}, bla_{NDM-1} and bla_{GIM} were detected by polymerase chain reaction (PCR).

Results: Of the 329 *P. aeruginosa*, majority of the isolates were resistant to imipenem (77.5%) followed by meropenem (64.7%). Of the 270 *P. aeruginosa* isolates tested, 149 (55.2%) isolates were found to be positive for MBL detection. Of the different samples, 57.8% (n=26) of *P. aeruginosa* isolated from blood were found to be positive for MBL production. Of the various MBL genes, bla_{IMP-1} (28.2%) was the most predominant gene detected followed by bla_{VIM-2} (18.8%), bla_{VIM-1} (16.1%), bla_{NDM-1} (9.4%),

bla_{IMP-2} (6.7%), bla_{SIM} (6.0%), bla_{SPM-1} (4.0%) and bla_{GIM} (1.3%) genes.

Conclusions: The high resistance of *P. aeruginosa* toward imipenem and meropenem and the high prevalence of bla_{IMP-1} and bla_{VIM-2} set the alarm on the increasing, perhaps the increased, carbapenem resistance. In addition to routine antibiotic susceptibility testings, our results emphasize the importance of both the phenotypic and genotypic MBL detection methods in routine practice for early detection of carbapenem resistance and to prevent further dissemination of this resistant pathogen.

Keywords: carbapenem resistance; metallo- β -lactamase genes; *Pseudomonas aeruginosa*.

Introduction

Pseudomonas aeruginosa, an opportunistic pathogen, is associated with nosocomial infections and causes various diseases including pneumonia, urinary tract infection, soft-tissue infection and sepsis [1]. Infections caused by multidrug-resistant (MDR) *P. aeruginosa* are associated with significant morbidity and mortality. A high level of intrinsic and acquired resistance to multiple antibiotics exhibited by *P. aeruginosa* makes it challenging to treat and limits the treatment options [2]. *Pseudomonas aeruginosa* exhibits almost all known resistance mechanisms; however, enzyme production is the major mechanism of acquired resistance, especially β -lactamase production [3]. The increased prevalence of extended-spectrum β -lactamase (ESBL)-producing *P. aeruginosa* led to the use of carbapenems. Carbapenems are the last choice of drug for the treatment of *P. aeruginosa* infections. However, the alarming increase in carbapenem resistance is a cause of serious concern in the treatment of *P. aeruginosa* infections [4]. The World Health Organization (WHO) has identified 12 most common bacteria that pose a challenge to human health. Among these, carbapenem-resistant *P. aeruginosa* was designated as one of the highly critical and poses a serious threat to patients who require ventilators and

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blood catheters [5]. β -lactamases are classified into four different classes: classes A, C and D act through a serine-based mechanism and metallo- β -lactamase (MBL), while class B requires a bivalent metal ion for its activity [6]. Of all these mechanisms, the emergence of *P. aeruginosa*-acquired MBL is the most worrisome and poses a serious threat during treatment and infection control [7]. Except for monobactams, MBL-producing strains can hydrolyze all other β -lactam antibiotics including penicillin, cephamycins, cephalosporin and carbapenems [8]. The MBL-producing *P. aeruginosa* strains carry co-resistance genes for other classes of antibiotics.

The MBL genes are present in mobile genetic elements such as plasmids, transposons, integrons or associated with insertion sequences with a tendency to spread within species and between different species [8]. Several types of MBL genes were identified in *P. aeruginosa*: (i) Verona integron-encoded metallo- β -lactamase (VIM), (ii) imipenemase (IMP), Seoul imipenemase (SIM), (iii) Germany imipenemase (GIM), (iv) São Paulo metallo- β -lactamase (SPM), (v) New Delhi metallo- β -lactamase (NDM) types. Of these, VIM and IMP are the most prevalent types of acquired MBLs [9, 10]. In 1991, MBL resistance was first reported in Japan; later it was reported in various countries including China, India, Taiwan, Singapore, Korea, Italy, France, Greece, Australia, Germany, Austria, Turkey, Bulgaria, Netherlands, Spain, Mexico, Colombia and USA [11]. Mortality due to MBL-producing *P. aeruginosa* ranged from 70% to 90% [11, 12].

When patients with severe infections caused by MBL-producing *P. aeruginosa* are treated with antibiotics, it often leads to poor clinical outcome. Thus, it is highly essential to detect MBL-producing *P. aeruginosa* as early as possible for the effective treatment of critically ill patients within clinical settings. The present study aimed to identify antibiotic susceptibility, phenotypic detection of MBL and to determine the prevalence of MBL genes in carbapenem-resistant *P. aeruginosa* isolated from different clinical samples.

Materials and methods

Bacterial strains

A total of 329 non-duplicate *P. aeruginosa* isolated from various clinical samples from the Beijing Friendship Hospital, Capital Medical University, Beijing, China and The Second Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan, China between September

2017 and March 2019 were included in this study. The isolates were identified as *P. aeruginosa* using the VITEK 2 system (bioMérieux, Craponne, France). Demographic data and other relevant details of patients from whom *P. aeruginosa* was isolated were collected from the hospital medical record department. The Institutional Ethical Board approved the study (IRB: 037-2017).

Susceptibility testing

Antibiotic susceptibility test for all *P. aeruginosa* isolates was performed by disc diffusion method on Mueller-Hinton agar (MHA) plates. The following antibiotics were used during the test: imipenem (10 μ g), meropenem (10 μ g), gentamicin (10 μ g), amikacin (30 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), norfloxacin (10 μ g), ciprofloxacin (5 μ g), piperacillin + tazobactam (100/10 μ g), colistin (10 μ g) and tigecycline (15 μ g) (Himedia, Mumbai, India). The results were interpreted as per the Clinical Laboratory Standard Institute (CLSI) guidelines [13].

Phenotypic detection of MBL production

All isolates that were resistant and intermediately resistant to imipenem by disc diffusion method were subjected to MBL production test by the combined disk method [14]. Briefly, overnight culture adjusted to 0.5 MacFarland standard was inoculated on to MHA plates. Two disks of 10 μ g imipenem (10 μ g) and imipenem (10 μ g) with 0.5 M ethylenediaminetetraacetic acid (EDTA) were placed 25 mm apart on the MHA plates. The plates were incubated at 37 °C for 16–18 h and observed for the zone of inhibition. A zone of ≥ 7 mm in the imipenem plus EDTA disc compared to imipenem alone disc was considered as positive for the presence of carbapenem resistance (MBL resistance).

Detection of MBL genes

Carbapenem-resistant isolates were subjected to polymerase chain reaction (PCR) for the detection of MBL genes including $\text{bla}_{\text{VIM-1}}$, $\text{bla}_{\text{VIM-2}}$, $\text{bla}_{\text{IMP-1}}$, $\text{bla}_{\text{IMP-2}}$, $\text{bla}_{\text{SPM-1}}$, bla_{SIM} , $\text{bla}_{\text{NDM-1}}$ and bla_{GIM} as described by Azimi et al. [15]. In-house *P. aeruginosa* isolates positive for all tested genes and confirmed earlier through sequencing were used as controls for PCR. The gene-specific primer sequence and their annealing temperature are presented in Table 1 [16–18]. DNA was extracted using a commercial genomic DNA extraction kit (Thermo Fisher Scientific, Waltham,

Table 1: Gene-specific primer sequence.

Gene	Primer sequence (5'-3')	Annealing temperature, °C
bla _{VIM-1}	F: 5'-AGTGGTGAGTATCCGACAG-3' R: 5'-ATGAAAGTGCCTGGAGAC-3'	53
bla _{VIM-2}	F: 5'-ATGTTCAAACTTTTGTAGTAAG-3' R: 5'-CTACTCAACGACTGAGCG-3'	52
bla _{IMP-1}	F: 5'-ACCGCAGCAGAGTCTTTGCC-3' R: 5'-ACAACCAAGTTTGCCTTACC-3'	55
bla _{IMP-2}	F: 5'-GTTTTATGTGTATGCTTCC-3' R: 5'-AGCCTGTCCCATGTAC-3'	51
bla _{SPM-1}	F: 5'-GCGTTTTGTTTGTGCTC-3' R: 5'-TTGGGGATGTGAGACTAC-3'	53
bla _{SIM}	F: 5'-TACAAGGGATTCCGCATCC-3' R: 5'-TAATGGCCTGTCCCATG-3'	52
bla _{NDM-1}	F: 5'-GGCGGAATGGCTCATCACGA-3' R: 5'-CGCAACACAGCCTGACTTTC-3'	56
bla _{GIM}	F: 5'-TCGACACACCTTGGTCTG-3' R: 5'-AACTTCCAACCTTGCCAT-3'	52

MA, USA). The PCR reaction mixture contained 12.5 μ L of ReadyMix™ Taq PCR Reaction Mix (Sigma-Aldrich, St. Louis, MO, USA), 2.5 μ L of the DNA (20 pg), and 0.5 μ M of each primer and nuclease-free water made up to 25 μ L. The PCR cycling conditions were as follows: initial denaturation at 96 °C for 10 min, followed by 30 cycles of 96 °C for 1 min, the specific annealing temperatures of the respective primers as presented in Table 1 for 1 min, and 72 °C for 1 min, and a final extension step at 72 °C for 10 min. After PCR, the amplicons were resolved in 1.2% (w/v) agarose gel electrophoresis and visualized under a ultraviolet (UV) transilluminator (BioRad, Hercules, CA, USA).

Statistical analysis

Descriptive statistics were performed to determine the frequencies. The chi-square (χ^2) test, Student's t-test and Pearson's correlation coefficient tests were performed to analyze the data using SPSS statistical software (IBM SPSS Inc., Chicago, IL, USA, Ver. 2015). A p-value of <0.05 was considered statistically significant.

Results

Patient characteristics

A total of 329 *P. aeruginosa* were isolated from 329 non-repetitive clinical samples collected from 307 patients

(mean age, 49.7 ± 6.7 years); of these, 171 (55.7%) were male and 136 (44.3%) were female. Various clinical samples included sputum (n=92, 28.0%), burnt wounds (n=71, 21.6%), bronchoalveolar lavage (BAL) (n=63, 19.1%), blood (n=45, 13.7%), pus (n=33, 10.0%) and others (n=25, 7.6%). Of the 307 patients, 171 (55.7%) patients were admitted in the intensive care unit (ICU) for various critical disease conditions (Table 2). Of the 307 patients, in 10 patients, two different samples were collected and in six patients, three different samples were collected.

Susceptibility testing

Of the 329 *P. aeruginosa*, majority of the isolates were resistant to imipenem (255, 77.5%) followed by meropenem (213, 64.7%), amikacin (209, 63.5%), gentamicin (201, 61.1%), norfloxacin (187, 56.8%), piperacillin/tazobactam (185, 56.2%), cefotaxime (184, 55.9%), ciprofloxacin (175, 53.2%), tigecycline (172, 52.3%), ceftazidime (167, 50.8%) and colistin (53, 16.1%). Compared to other samples, a higher number of *P. aeruginosa* (71, 77.2%) isolated from sputum samples were found to be resistant to imipenem ($p > 0.05$). All isolates that were resistant to meropenem were found to be resistant to imipenem. The correlation

Table 2: Patient demographics and clinical condition.

Description	No. of patients/ samples, %
Patients (n = 307)	
Male	171 (55.7%)
Female	136 (44.3%)
Age (mean $\pm$ SD)	49.7 ± 6.7 years
In ICU	171 (55.7%)
In ward	136 (44.3%)
Under ventilator support	89 (28.3%)
On catheter	56 (18.2%)
Prior antibiotic therapy	237 (77.2%)
Clinical condition	
Respiratory disorder	127 (41.4%)
Burnt wound	80 (26.1%)
Septicemia	72 (23.5%)
Diabetes	19 (6.2%)
Urinary tract infection	9 (2.9%)
Clinical samples (n = 329)	
Sputum	92 (28.0%)
Burnt wound	71 (21.6%)
Bronchoalveolar lavage	63 (19.1%)
Blood	45 (13.7%)
Pus	33 (10.0%)
Urine	25 (7.6%)

ICU, intensive care unit; SD, standard deviation.

coefficient showed that there was a significant association between the *P. aeruginosa* isolated from patients with respiratory diseases and imipenem resistance ($r=0.98$, $p=0.029$). Thirty-two (9.7%) isolates were found to be susceptible to all the antibiotics tested. A total of 207 (62.9%) isolates were found to be MDR strains as they were intermediately or fully resistant to at least three different classes of antibiotics. None of the *P. aeruginosa* isolated from blood samples was resistant to colistin (Table 3).

Phenotypic detection of MBL production

All the isolates that were resistant (255, 77.5%) and intermediately resistant (15, 4.6%) to imipenem were subjected to MBL detection by the combined disk method. Of the 270 *P. aeruginosa* isolates tested, 149 (55.2%) isolates were found to be positive for MBL detection. Of the different samples, 57.8% (26/45) of *P. aeruginosa* isolated

from blood were found to be positive for MBL, followed by *P. aeruginosa* isolated from pus (18/33, 54.5%), sputum (41/92, 44.6%), burnt wound (31/71, 43.7%), BAL (24/63, 38.1%) and urine (9/25, 36.0%). The MBL was predominantly detected in *P. aeruginosa* isolated from male (83, 55.7%) compared to female (66, 44.3%) patients (Table 4). The presence of MBL among *P. aeruginosa* isolated from different samples did not differ significantly ($p>0.05$).

Detection of MBL genes

All the 149 *P. aeruginosa* that were positive for carbapenem resistance were subjected to MBL gene detection by PCR. Of the various MBL genes tested, $\text{bla}_{\text{IMP-1}}$ (41, 28.2%) was the most common gene detected from the isolates followed by the $\text{bla}_{\text{VIM-2}}$ (28, 18.8%), $\text{bla}_{\text{VIM-1}}$ (24, 16.1%), $\text{bla}_{\text{NDM-1}}$ (14, 9.4%), $\text{bla}_{\text{IMP-2}}$ (10, 6.7%), bla_{SIM} (9, 6.0%), $\text{bla}_{\text{SPM-1}}$ (6, 4.0%) and bla_{GIM} (2, 1.3%) genes. The presence of the $\text{bla}_{\text{IMP-1}}$ gene was significantly higher among the isolates tested ($p=0.023$). Of the various combinations identified, the presence of the $\text{bla}_{\text{IMP-1}}$ gene along with $\text{bla}_{\text{IMP-2}}$ was the most common combination of genes present among the isolates (Table 5). Fourteen (9.4%) isolates did not amplify any of the genes tested.

Discussion

Pseudomonas aeruginosa infections are effectively treated by carbapenems; however, resistance toward these antibiotics spread across hospitals due to the extensive use of antibiotics [19]. Intrinsic resistance among *P. aeruginosa*

Table 3: Antibiotic susceptibility testing.

Antibiotics	Susceptible	Intermediate	Resistant
Imipenem	59 (17.9%)	15 (4.6%)	255 (77.5%)
Meropenem	84 (25.5%)	32 (9.7%)	213 (64.7%)
Amikacin	72 (21.9%)	48 (14.6%)	209 (63.5%)
Gentamicin	94 (28.6%)	34 (10.3%)	201 (61.1%)
Norfloxacin	121 (36.8%)	21 (6.4%)	187 (56.8%)
Piperacillin/tazobactam	117 (35.6%)	27 (8.2%)	185 (56.2%)
Cefotaxime	91 (27.1%)	54 (16.4%)	184 (55.9%)
Ciprofloxacin	122 (37.1%)	32 (9.7%)	175 (53.2%)
Tigecycline	119 (36.2%)	38 (11.6%)	172 (52.3%)
Ceftazidime	114 (34.7%)	48 (14.6%)	167 (50.8%)
Colistin	227 (69.0%)	53 (14.9%)	53 (16.1%)

Table 4: Phenotypic detection of MBL-producing *P. aeruginosa*.

Antibiotics	Sputum (n=92)	Burnt wound (n=71)	BAL (n=63)	Blood (n=45)	Pus (n=33)	Urine (n=25)
Imipenem	86 (93.5%)	57 (80.3%)	45 (71.4%)	29 (64.4%)	21 (63.6%)	17 (68.0%)
Meropenem	75 (81.5%)	65 (91.5%)	33 (52.4%)	20 (44.4%)	12 (36.4%)	8 (32.0%)
Amikacin	41 (44.6%)	52 (73.2%)	51 (80.9%)	22 (48.9%)	24 (72.7%)	19 (76.0%)
Gentamicin	42 (45.7%)	51 (71.8%)	47 (74.6%)	21 (46.7%)	26 (78.8%)	14 (56.0%)
Norfloxacin	56 (60.9%)	41 (57.7%)	26 (41.3%)	28 (62.2%)	21 (63.6%)	15 (60.0%)
Piperacillin/tazobactam	48 (52.2%)	61 (85.9%)	24 (38.1%)	21 (46.7%)	17 (51.5%)	14 (56.0%)
Cefotaxime	54 (58.7%)	40 (56.3%)	32 (50.8%)	23 (51.1%)	23 (69.7%)	12 (48.0%)
Ciprofloxacin	42 (45.7%)	53 (74.6%)	38 (60.3%)	19 (42.2%)	14 (42.4%)	9 (36.0%)
Tigecycline	36 (39.1%)	41 (57.7%)	44 (69.8%)	18 (40.0%)	17 (51.5%)	16 (64.0%)
Ceftazidime	71 (77.2%)	36 (50.7%)	21 (33.3%)	22 (48.9%)	9 (27.3%)	8 (32.0%)
Colistin	18 (19.6%)	15 (21.1%)	6 (9.5%)	0 (0.0%)	9 (27.3%)	5 (20.0%)

BAL, bronchoalveolar lavage; MBL, metallo- β -lactamase.

Table 5: Distribution of MBL genes among *P. aeruginosa*.

Gene combination	No. of isolates
<i>bla</i> _{IMP-1}	41 (27.5%)
<i>bla</i> _{VIM-2}	28 (18.8%)
<i>bla</i> _{VIM-1}	24 (16.1%)
<i>bla</i> _{NDM-1}	14 (9.4%)
<i>bla</i> _{IMP-2}	10 (6.7%)
<i>bla</i> _{SIM}	9 (6%)
<i>bla</i> _{SPM-1}	6 (4%)
<i>bla</i> _{GIM}	2 (1.3%)
<i>bla</i> _{IMP-1} , <i>bla</i> _{IMP-2}	6 (4.0%)
<i>bla</i> _{VIM-1} , <i>bla</i> _{VIM-2}	4 (2.7%)
<i>bla</i> _{IMP-1} , <i>bla</i> _{NDM-1}	4 (2.7%)
<i>bla</i> _{SIM} , <i>bla</i> _{IMP-2}	2 (1.3%)
<i>bla</i> _{NDM-1} , <i>bla</i> _{VIM-1} , <i>bla</i> _{IMP-1}	2 (1.3%)
<i>bla</i> _{NDM-1} , <i>bla</i> _{GIM} , <i>bla</i> _{IMP-1}	2 (1.3%)
<i>bla</i> _{NDM-1} , <i>bla</i> _{VIM-1} , <i>bla</i> _{IMP-1} , <i>bla</i> _{IMP-2}	1 (0.7%)

MBL, metallo- β -lactamase.

leads to MDR and is associated with high mortality. This study reported a high level of carbapenem resistance among *P. aeruginosa* isolated from various clinical samples. Although not significant, *P. aeruginosa* (77.2%) isolated from sputum samples were found to be resistant to imipenem ($p > 0.05$). The majority of our isolates were from sputum samples (28.0%) and the majority of our sputum samples (72.4%) were from patients with respiratory diseases. A significant association between the *P. aeruginosa* isolated from patients with respiratory diseases and imipenem resistance ($r = 0.98$, $p = 0.029$) was found in this study. In our hospital, imipenem is one of the most commonly used antibiotics as first-line therapy for most of the cases with pseudomonas infection, especially for respiratory diseases and patients who are on ventilator support. An overexposure of this drug could possibly be the reason for this higher rate of imipenem resistance among our isolates.

A wide range of resistant mechanisms make *P. aeruginosa* swiftly change to the selective environmental pressure and makes it resistant to several classes of antibiotics. In this study, 77.5% of the isolates were found to be resistant to imipenem, which corroborates to that reported from Egypt (78.3%) [20] and China (73.3%) [21]. Compared to other studies from the Asia-Pacific region including Iran (25.2%) [22], China (17.1%, 43.3%) [23, 24], Japan (28.5%) [25] and Taiwan (16%) [26], this study reported a much higher resistance toward imipenem. Another study from Iran reported a resistance of 98.8% [15]. In a review article, Hong et al. presented an overview of the epidemiology and molecular characteristics of MBL-producing *P. aeruginosa*.

The study which analyzed several publications from Asia, Europe, America and Africa reported imipenem resistance among *P. aeruginosa* ranging from 8% to 66% [9]. In this study, meropenem resistance was the second most common resistance reported (64.7%) among our isolates. In addition to imipenem resistance, Hong et al. reported meropenem resistance ranging from 8% to 57% [9]. In the aforementioned study, which covers multiple countries of all regions, the reported imipenem and meropenem resistance ranges among *P. aeruginosa* do not exceed the rate reported in this study [9]. This implies that imipenem and meropenem resistance among our isolates was higher than that reported worldwide and needs special attention. In addition to the overuse of imipenem and meropenem in our hospital settings, the presence of resistant genes on mobile genetic elements may have also contributed to the spread of resistance within our isolates [8]. In our study, 62.9% of the isolates were found to be MDR strains, which was higher than that reported from Brazil (37%) and Asia (42.8%) [27, 28].

In this study, 55.2% of the isolates were found to be MBL producers. A study from Iran, which included *P. aeruginosa* isolated from burnt wound, reported a lower rate (43.7%) of MBL producers compared to this study [15]. Similarly, another study from Iran also reported a lower rate (37.7%) of MBL producers among their clinical isolates of *P. aeruginosa* [29]. Hong et al. in a review article analyzed reports with varied carbapenem resistance rates. Compared to this study, the review article reported lower carbapenem-resistant isolates from South and Southeast Asia including Philippines (31.1%), Singapore (23.3%), Thailand (28.7%), Vietnam (46.7%) and India (32%); Oceanic including Australia (16%) and New Zealand (10.3%); East Africa including Kenya (13.7%); and North America including Canada (3.3%) and the United States (20%). A much higher rate was reported from Russia (75.3%) and Costa Rica (63.1%) [9]. A meta-analysis from Iran, which included 14 publications on *P. aeruginosa* isolated from burn patients, reported a pooled prevalence rate of 76.8% carbapenem-resistant *P. aeruginosa*, which is much higher than that reported in this study [30]. However, a study from China, which included *P. aeruginosa* isolated from cystic fibrosis patients, reported that 56.25% of their isolates were found to be MBL producers, which is comparable to that reported in this study [21]. Although not significant, a relatively high number (41, 27.52%) of *P. aeruginosa* isolated from sputum samples were found to be MBL producers. However, in terms of percentage, *P. aeruginosa* isolated from blood samples yielded a higher rate (57.8%) of MBL producers. *Pseudomonas aeruginosa* isolated from male

(55.7%) patients were found to be the predominant MBL producers.

Most of the MBL genes are located in the integrons within mobile genetic elements and are responsible for the dissemination of antibiotic resistance through horizontal gene transfer. In the past decade, the emergence and dissemination of the newly identified bla_{NDM} and bla_{AIM} and the most prevalent bla_{IMP}, bla_{VIM}, bla_{SIM} and bla_{GIM} MBL genes have been widely reported around the world [20]. In this study, bla_{IMP-1} (28.2%) was the most common gene detected; bla_{VIM-2} (18.8%) was the second most common gene detected, followed by bla_{VIM-1} (16.1%). Similar to this study, a study from Iran which included MBL-producing *P. aeruginosa* reported that bla_{IMP} (24.65%) was the predominant gene detected followed by bla_{VIM} (11.5%) [29]. Similarly, a study from Japan which included 180 *P. aeruginosa* reported that bla_{IMP-1} was the predominant gene detected (116/180 isolates) followed by bla_{VIM-2} (63/180 isolates) [16]. While a meta-analysis from Iran which included *P. aeruginosa* from burn patients reported that the pooled prevalence of bla_{VIM} was 21.4% and of bla_{IMP} was 13.1%, the reported rates were higher than in this study [30]. A study from Iran also reported that bla_{VIM} (17.5%) was the most common gene followed by bla_{IMP} (15.6%) [15]. A study from China that included 63 MBL-producing *P. aeruginosa* reported that bla_{VIM} was the most common gene but with a higher rate (84.1%) and bla_{IMP} was the second most common gene (76.1%), however, with higher rates than those reported in this study [21]. A study from Iran that included 19 MBL-producing *P. aeruginosa* reported that 31.5% of their isolates carried the bla_{VIM} gene and 10.5% carried bla_{IMP}, which was higher than that reported in this study [22]. In contrast to our study, a study from Egypt reported that none of their *P. aeruginosa* was positive for the bla_{VIM} gene [20]. In the present study, 9.4% of our isolates were positive for the bla_{NDM-1} gene, which is lower than that reported from India (27%) [31]. A study from Bahrain reported a lower rate of bla_{NDM-1} (2.5%) than that reported in this study [32]. In the present study, the bla_{IMP-1} gene was significantly higher among the isolates tested ($p=0.023$). The bla_{IMP-1} gene along with bla_{IMP-2} was the most common combination found in this study. There was no significant difference in the presence of MBL genes with that of hospital settings, including ICU/in-ward patients, patients under ventilator and burnt wound patients ($p>0.05$). Fourteen (9.4%) isolates did not amplify any of the genes tested, and these isolates could possibly carry other resistant gene variations that were not tested in this study. A study to test more resistant gene variations could possibly reveal the resistant genes associated with these isolates. The present study suggests that with the predominance

of the bla_{IMP-1} gene, bla_{VIM} is also increasing in our region. In addition, the high rate of the bla_{NDM-1} gene signifies its spread and emphasizes the need for continuous monitoring for better patient management and infection control.

Conclusions

In conclusion, the relatively high resistance of *P. aeruginosa* toward imipenem and meropenem and the high prevalence of bla_{IMP-1} and bla_{VIM-2} set an alarm on the increasing, perhaps the increased, carbapenem resistance. In addition to routine antibiotic susceptibility testings, our results emphasize the importance of both the phenotypic and genotypic MBL detection methods in routine practice for early detection of carbapenem resistance and to prevent further dissemination of this resistant pathogen.

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