Original Article

Detection of Different Types of Metallo-β-Lactamases among *Pseudomonas aeruginosa* Isolates Obtained from Intensive Care Unit Patients

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Introduction: *Pseudomonas aeruginosa* is a serious challenge for antimicrobial therapy, due to chromosomal mutations or intrinsic resistance to various antimicrobial agents, such as Metallo-β-Lactams (MBL). This study aimed to investigate the prevalence of β-lactamases encoding genes among *P. aeruginosa* strains isolated from intensive care unit (ICU) patients by phenotypic and multiplex PCR methods. **Methods:** A total of 48 non-duplicate strains of *P. aeruginosa* were collected from different clinical specimens of patients hospitalized in ICU wards of a teaching hospital in Isfahan, Iran. Susceptibility test was performed by disk diffusion method. All meropenem resistant strains were subjected to modified Hodge test (MHT) for detection of carbapenemases. Multiplex PCRs were performed to detect β-lactam-resistant *P. aeruginosa* isolates. **Results:** In disk diffusion method, *P. aeruginosa* strains showed the most (97.9%) resistance against imipenem and meropenem and the least (45.8%) against colistin. Thirty-six (75%) out of the 48 isolates were multidrug resistant. PCR amplification of β-lactamase genes showed the presence of *bla*$_{VIM}$ genes in 7 (14.6%) and *bla*$_{IMP}$ in 15 (31.3%) strains. Also, *bla*$_{SME, SPM, GIM, AIM and NDM}$ genes were not observed in any of the strains. We only found a statistically significance difference between the presence of *bla*$_{IMP}$ gene and multidrug-resistant (MDR) positivity and source of specimen (p=0.009 and 0.002, respectively). **Conclusion:** Rapid and reliable identification of MBLs appears to be necessary for effective treatment of related infections. Besides, our results may provide useful perception to make a more appropriate choice of antibiotics, which may put a stop to carbapenem-resistant infections. *J Med Microbiol Infec Dis, 2014, 2 (2): 84-90.*

Keywords: *Pseudomonas aeruginosa*, PCR, Carbapenem, Beta-lactamase.

INTRODUCTION

Pseudomonas aeruginosa is widely known as an opportunistic invader rather than a cause of primary infection in healthy subjects [1]. *P. aeruginosa* causes approximately 10% of nosocomial infections with a high mortality rate. The complete sequencing of a wild-type *P. aeruginosa* strain has provided useful information on not only to its pathogenicity, but also to its potential for resistance to many antibiotic agents and the development of increased multidrug resistance (MDR) in healthcare settings [2, 3].

Currently, the emergence of carbapenemase producing strains is noticeable among *Enterobacteriaceae*, such as *Pseudomonas* spp. [4]. Carbapenems are drugs of choice for the treatment of infections caused by penicillin- or cephalosporin- resistant Gram-negative and nonfermenting bacilli, such as *P. aeruginosa* and *Acinetobacter* spp. [1].

The Metallo-β-lactamase (MBL) producing strains are often resistant to aminoglycosides and fluoroquinolones, but susceptible to polymyxins. MBL producing *P. aeruginosa* strains are the cause of several nosocomial outbreaks in tertiary centers worldwide, exhibiting the necessity of an appropriate infection control strategy. Also, these isolates cause serious infections, such as septicemia and pneumonia and are associated with failure of treatment with carbapenems [5, 6].

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Carbapenem resistance can occur, and it involves different mechanisms in *P. aeruginosa*, such as reduced outer membrane permeability, increased energy-dependent efflux, porin down regulation, and production of carbapenem-hydrolyzing enzymes [1]. In addition, most of the carbapenemase genes are plasmid carried and easily transferable between various bacterial species and genera [7].

Carbapenems (meropenem and imipenem) are effective against Gram-negative MDR strains [8]. Current data propose that carbapenem resistance is increasing among isolates of *P. aeruginosa* [3]. MBL producing strains hydrolyze all carbapenems, and deficiency of these enzymes can result in resistance to imipenem and reduced sensitivity to meropenem [9].

In the last decade, various classes of β-lactamases (A, B, and D) have been identified in *P. aeruginosa* [10]. The carbapenemases found, are mostly different types of MBLs, such as *AIM, SME, GIM, IMP, NDM, SPM*, and *VIM*. The frequency of these types has increased in *P. aeruginosa* worldwide [1].

Nosocomial outbreaks caused by MBL-producing *P. aeruginosa* have been reported in many countries worldwide [11]. Carbapenemases that hydrolyze carbapenems and make them inactive have been progressively reported in Asia, Europe, Canada, and the United States [12].

Various current studies from different parts of our country revealed an increased frequency of MBL-producing *P. aeruginosa* in different hospital units [13].

Several phenotypic and genotypic tests have been carried out to confirm carbapenem resistant cases. Among phenotypic tests, the modified Hodge-test (MHT) is an easy test to perform, but it is not recommended for non-fermenters [5, 12].

Identification of genes coding for carbapenem resistance by PCR, usually provides reliable and accurate results, but application of this method is limited to clinical laboratories due to the cost [1].

Single or multiplex PCR assays require expert knowledge of carbapenemases epidemiology and these methods are typically focused only on prevalent carbapenemase genes [14].

Assessment of phenotypic and genotypic characteristics of these isolates would be necessary for understanding the resistance mechanisms and their potential spread. We designed this study to determine the prevalence of MBL-producing *P. aeruginosa* (the presence of *AIM, SME, GIM, IMP, NDM, SPM*, and *VIM*) among *P. aeruginosa* strains isolated from intensive care unit (ICU) patients in AL-Zahra Hospital of Isfahan, Iran.

MATERIAL AND METHODS

Bacterial identification. In a retrospective cross-sectional study, a total of 48 non-duplicate isolates of *P. aeruginosa* were collected from clinical specimens of patients hospitalized in ICU wards of Al-Zahra hospital of Isfahan City, Iran, from March 2012 to April 2013. These isolates were obtained from culture of specimens from tracheal aspirate, blood, urine, wound, catheter, and peritoneal fluid of the patients.

Bacteria were identified as *P. aeruginosa* by biochemical tests or based on the following characteristics: Gram-negative bacilli, citrate positive, non-fermentative, TSI Alk/Alk, motile, H_2S negative, urease negative, oxidase positive, and catalase positive. PCR amplification was performed to verify these isolates with *P. aeruginosa* specific primers (primer sequence: gyr B-F: 5'-CCTGACCATCCGTCGCCACAAC-3', *gyr B-R*: 5'-CGCAGCAGGATGCCGACGCC-3') with product size 222 bp [15]. The confirmed isolates were kept at -70°C. Standard strain *P. aeruginosa* ATCC 27853 was used as control [3].

Antimicrobial susceptibility testing. Antibiotic susceptibility testing was performed for all collected *P. aeruginosa* isolates by disk diffusion method on Mueller-Hinton agar (Merck, Germany). Susceptibility was defined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. Antibiogram disks containing meropenem (MEM: 10 µg), imipenem (IPM: 10 µg), ceftazidime (CAZ: 30 µg), cefepime (FEP: 30 µg), amikacin (AK: 30 µg), ciprofloxacin (CIP: 5 µg), colistin sulphate (10 µg), ampicillin/sulbactam (20 µg), and cefotaxime (CTX: 30 µg) (MAST, Bootle, Merseyside, UK), were used to test antimicrobial susceptibility. If an isolate was resistant to three or more classes of antibiotics (including penicillins/cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones), it was regarded as MDR [16]. *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as reference strains in susceptibility test [3, 17].

Modified Hodge test. The MHT was performed for all of the meropenem resistant isolates (*i.e.* inhibition zone: 16-21 mm) for detection of carbapenemases. Briefly, a 1/10 dilution of an inoculum of the indicator organisms, adjusted to a 0.5 McFarland turbidity standard, was used to inoculate by swabbing the surface of Mueller-Hinton agar (Hi-Media, India) plates. Then, a meropenem disk (MEM: 10 µg; MAST, Bootle, Merseyside, UK) was placed on each plate. An overnight grown single colony of the isolate was inoculated onto the plates. The presence of an inhibition zone due to carbapenemase production by the test strain was considered as positive. Besides, combined-disk (CD) tests with boromic acid and EDTA were performed for detection of class A carbapenemases and MBLs, respectively. In MBL-producing strains, the difference of inhibition zone between carbapenem disk and carbapenem-EDTA disk was ≥7 mm) [5, 18, 19].

Extraction of total DNA. For molecular identification, total DNA content of each isolate was extracted using a modified boiling method. Briefly, a single colony from a 16-hour culture on the nutrient agar (Hi-Media, India) was suspended in 50 µl of TES buffer [50 mM Tris hydrochloride (pH 8.0), 5 mM EDTA, 50 mM NaCl], and the suspension was heated at 95°C for 10 min and placed at room temperature for 5 min. Then it was centrifuged at 14,000 rpm for 10 min at 4°C. DNA-containing supernatant

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was transferred to new sterile DNase free-RNase free microtubes [20].

Rapid detection of *AIM, SME, GIM, IMP, NDM, SPM,* and *VIM* Genes by Multiplex PCR. Multiplex PCR was performed for amplification of *AIM*, *SME*, *GIM*, *IMP*, *NDM*, *SPM*, and *VIM* genes of *P. aeruginosa*, using the primers listed in Table 1 according to the previous protocols [21]. Two multiplex reactions were designed; one was detection of bla_{IMP}, bla_{VIM} and bla_{SME}, and the other was detection of bla_{NDM}, bla_{AIM}, bla_{SPM}, and bla_{GIM}.

Two µL of total DNA was subjected to multiplex PCR in a 50-µL reaction mixture. The mix for the detection of bla_{IMP}, bla_{VIM}, and bla_{SME} genes contained 1× PCR buffer [10 mmol/L Tris–HCl (pH 8.3), 50 mmol/L KCl], 1.5 mmol/L $MgCl_2$, 0.125 mmol/L of each deoxynucleotide triphosphate, 10 µmol/L of each primer, and 2 U of Taq polymerase (Fermentas R, Korea). The mix for the detection of bla_{NDM}, bla_{AIM}, and bla_{SPM}, bla_{GIM} genes contained 1× PCR buffer [10 mmol/L Tris–HCl (pH 8.3), 50 mmol/L KCl], 3 mmol/L $MgCl_2$, 0.125 mmol/L of each deoxynucleotide triphosphate, 10 µmol/L of each primer, 3 µL of dimethyl sulfoxide, and 2.5 U of Taq polymerase.

Amplification was carried out using the following thermal cycling conditions: initial DNA denaturation at 94°C for 10 min, followed by 36 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 40 sec, and extension at 72°C for 50 sec, followed by final extension at 72°C for 5 min. Agarose gel electrophoresis of the amplified DNA product with 100 bp size marker (Fermentas R, Korea) was performed for one hour at 80 V in a 2% agarose gel in 1× TAE buffer [40 mmol/L Tris–HCl (pH 8.3), 2 mmol/L acetate, 1 mmol/L EDTA] containing 0.05 mg/L ethidium bromide to detect the specific band [21]. Primers were purchased from Metabion (Munich, Germany) and other molecular materials purchased from Cinnagen, Iran. Reference strain *P. aeruginosa* ATCC 27853 was used as the bla_{MBL} gene's negative control strain.

Statistical analysis. Statistical analyses were performed using the statistical software SPSS version 18.0 for Windows (SPSS Inc., Chicago, IL). The diagram was plotted using Excel software version 2013 (Microsoft Corporation, USA). The intermediate level of susceptibility was considered as resistance in data analysis.

Continuous variables were presented as mean ± standard deviation (SD) and compared using the Student's t-test, or expressed as the median and range. Categorical variables were compared using Chi-square test or Fisher exact test if the expected values were less than 10. All tests were two-tailed and $p<0.05$ was considered statistically significant.

Table 1. Oligonucleotides used in this study

Primer[a]	Sequence (5′–3′)[b]	Gene	Product size (bp)[c]
IMP-F	GGAATAGAGTGGCTTAAYTCTC	bla_{IMP}	232
IMP-R	GGTTTAAYAAAACAACCACC		
SPM-F	AAAATCTGGGTACGCAAACG	bla_{SPM}	271
SPM-R	ACATTATCCGCTGGAACAGG		
AIM-F	CTGAAGGTGTACGGAAACAC	bla_{AIM}	322
AIM-R	GTTCGGCCACCTCGAATTG		
VIM-F	GATGGTGTTTGGTCGCATA	bla_{VIM}	390
VIM-R	CGAATGCGCAGCACCAG		
SME-F	ACTTTGATGGGAGGATTGGC	bla_{SME}	551
SME-R	ACGAATTCGAGATCACCAG		
GIM-F	TCGACACACCTTGGTCTGAA	bla_{GIM}	477
GIM-R	AACTTCCAACTTTGCCATGC		
NDM-F	GGTTTGGCGATCTGGTTTTC	bla_{NDM}	621
NDM-R	CGGAATGGCTCATCACGATC		

[a] F, sense primer; R, antisense primer
[b] Y=C or T
[c] Nucleotide numbering begins at the initiation codon of genes

RESULTS

Demographic characteristics of carriers of *P. aeruginosa.* The medical records of the 48 index patients were reviewed. The mean age of the patients was 49.5±19.2 years, with a male to female ratio of 1.82:1. Seventeen (35.4%) patients were female and 31 (64.6%) were male. The samples were urine (n=19, 39.5%), blood (n=12, 25%), tracheal aspirate (n=11, 22.9%), wound (n=4, 8.3%), catheter (n=1, 2.08%), and peritoneal fluid (n=1, 9%). The most common underlying diseases were urinary and blood infections.

Antimicrobial susceptibility of clinical strains. The antibiotic resistance of the strains are shown in Figure 1. In the disk diffusion method, *P. aeruginosa* strains showed the most (97.9%) resistance against imipenem and meropenem and the least (45.8%) against colistin. Thirty-six (75%) out of the 48 isolates were MDR. Chi-square analysis showed no significant relationship between the MDR positive and MDR negative isolates ($p=0.99$) in terms of gender. Also, MDR and non-MDR isolates did not show any statistically significant difference in terms of source of specimen ($p=0.85$) and mean age ($p=0.52$). Frequency of MDR and non-MDR isolates as well as demographic characteristics of patients are listed in Table 2.

Testing of Bacterial isolates for the presence of *AIM, SME, GIM, IMP, NDM, SPM,* and *VIM* genes. All of the 48 *P. aeruginosa* isolates were verified by amplification of the *gyrB* gene. PCR screening was performed for all strains.

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bla$_{IMP}$ and *bla*$_{VIM}$ genes were detected in 15 (31.3%) and 7 (14.6%) isolates, respectively. In one isolate of *P. aeruginosa* from urine, both *bla*$_{IMP}$ and *bla*$_{VIM}$ genes were detected. Positive controls of *bla*$_{IMP}$ and *bla*$_{VIM}$ (used individually or combined) produced expected bands and confirmed the specificity of the PCR primers used in simplex and multiplex PCR methods. Expected fragments were seen in multiplex PCR method (Figures 2-4). MHT or CD tests were positive for isolates with *bla*$_{IMP}$ or *bla*$_{VIM}$ genes, and all of them were MDR. Fisher exact test showed a statistically significant difference between the presence of *bla*$_{IMP}$ gene and MDR positivity (p=0.009). Frequency of *P. aeruginosa* isolates, having *bla*$_{VIM}$ and *bla*$_{IMP}$ genes, was determined based on demographic characteristics of patients (Table 3). Prevalence of *bla*$_{IMP}$ and *bla*$_{VIM}$ genes in male and female patients were 32.3%, 16.1%, and 29.4%, 11.8%, respectively. Chi-square analysis did not show any statistically significance difference between males and females in terms of the presence of these genes (p>0.05). The mean age of patients with *bla*$_{IMP}$ gene was lower than other patients, but this difference was not statistically significant (p=0.84). Ten (52.6%) out of 19 urine samples contained *bla*$_{IMP}$. The Fisher exact test showed a significant difference between the source of specimen and the presence of *bla*$_{IMP}$ gene (p=0.002). We could not find any statistically significant difference between the presence of *bla*$_{VIM}$ gene and MDR positivity (p=0.11), mean age (p=0.64), and source of the specimen (p=0.15). Also, both multiplex and simplex PCR amplifications showed that all of the isolates were negative for *bla*$_{GIM}$, *bla*$_{SME}$, *bla*$_{SPM}$, *bla*$_{NDM,}$ and *bla*$_{AIM}$ genes.

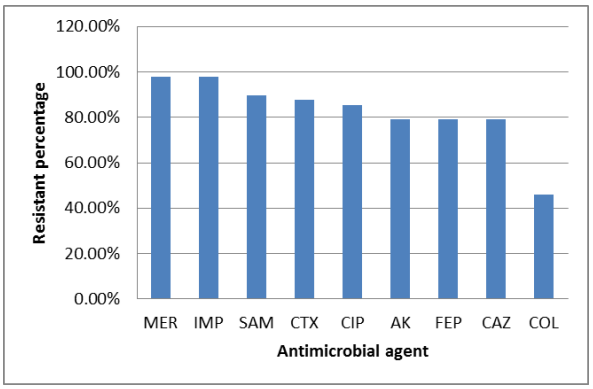


Fig. 1. Antimicrobial resistance rates among *P. aeruginosa* isolates

MER= Meropenem; IMP= ImipeneM; SAM= Ampicillin/sulbactam; CTX= Cefotaxime; CIP= Ciprofloxacin; AK= Amikacin; FEP= Cefepime; CAZ= Ceftazidime; COL= Colistin

Table 2. Frequency of MDR and non-MDR isolates versus demographic characteristics of patients

Parameters	MDR No. (%)	Non-MDR No. (%)	*P*-value
Age, mean ± SD (y)	49.1±17.4	50.4±24.8	0.85
Sex			
Male	23 (74.2)	8 (25.8)	0.99
Female	13 (76.5)	4 (23.5)	
Isolated specimen site			
Urine	1 (100)	0 (0)	
Catheter	14 (73.7)	5 (26.3)	
Tracheal aspirate	6 (54.5)	5 (45.5)	0.52
Blood	10 (83.3)	2 (16.7)	
Wound	4 (100)	0 (0)	
Peritoneal fluid	1 (100)	0 (0)	

Table 3. Results for ß-lactamase genes in *P. aeruginosa* isolates versus demographic characteristics of patients

Parameters	No. (%) of positive isolates for *bla*$_{IMP}$ gene	*P*-value	No. (%) of positive isolates for *bla*$_{VIM}$ gene	*P*-value
Age, mean ± SD (y)	55.7±11.2	0.13	46.3±11.5	0.64
MDR	15 (14.7)	0.009	7 (19.4)	0.11
Non MDR	0 (0)		0 (0)	
Sex				
Male	10 (32.3)	0.84	8 (25.8)	0.99
Female	5 (29.4)		4 (23.5)	
Isolated specimen site				
Urine	10 (52.6)		4 (21.1)	
Catheter	0 (0)		1 (100)	
Tracheal aspirate	2 (18.2)	0.002	2 (18.2)	0.15
Blood	0 (0)		0 (0)	
Wound	3 (75)		0 (0)	
Peritoneal fluid	0 (0)		0 (0)	

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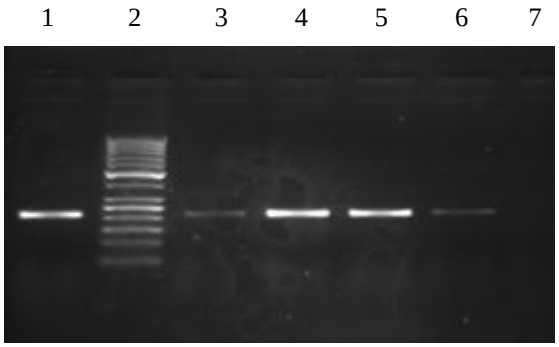


Fig. 2. Gel electrophoresis of multiplex PCR products after amplification with specific primers for *bla*$_{IMP}$ gene (232 bp)

Lane 1, *bla*$_{IMP}$ (232 bp) positive control; lane 2, the 50 bp Ladder; lanes 3-6, clinical isolates for *bla*$_{IMP}$ gene; lane 7, negative control.

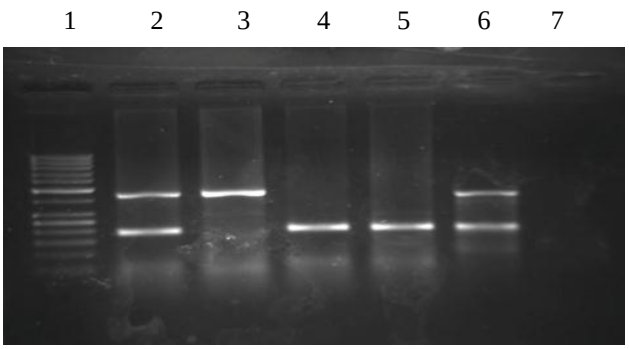


Fig. 3. Gel electrophoresis of multiplex PCR products after amplification with specific primers for *bla*$_{IMP}$ gene (232 bp) and *bla*$_{VIM}$ gene (391 bp)

Lane 1, the 50 bp Ladder; lane 2, *bla*$_{IMP, VIM}$ positive control; lane 3, positive clinical isolate for *bla*$_{VIM}$ gene; lanes 4-5, positive clinical isolates for *bla*$_{IMP}$ gene; lane 6, positive clinical isolate for *bla*$_{IMP}$ and *bla*$_{VIM}$ genes; lane 7, negative control.

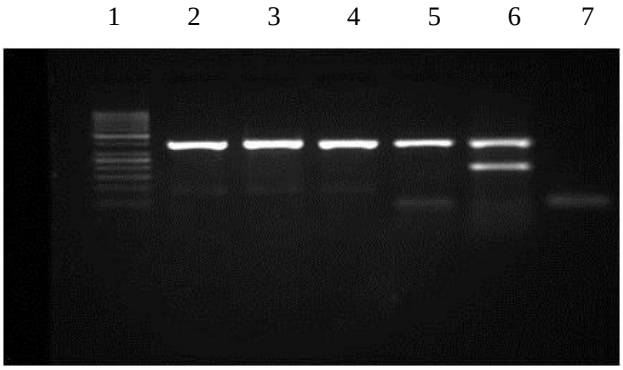


Fig. 4. Gel electrophoresis of multiplex PCR products after amplification with specific primers for *bla*$_{VIM}$ gene (391 bp)

Lane 1, the 50 bp Ladder; lanes 2-5, clinical isolates for *bla*$_{VIM}$ gene; lane 6, *bla*$_{VIM}$ and *bla*$_{IMP}$ (232 bp) positive control; lane 7, negative control.

DISCUSSION

MDR Gram-negative bacteria like *P. aeruginosa* is one of the most important problems in the treatment of hospitalized patients [16]. Carbapenems are an appropriate choice for the treatment of infections with these bacteria [1]. Carbapenem-resistant *P. aeruginosa* has appeared as a global serious pathogen, but its prevalence varies by geographic region, specimen origin, patient's age, clinical setting, and selective pressure caused by broad spectrum antibiotics [22].

The rate of resistance to carbapenems in *Pseudomonas* isolates ranged from 7.4% to 35.4% in the US during 2010. This rate varied between 18.1% in 2011 and 10.2% in 2013 among all *P. aeruginosa* isolates in ICUs of Taiwan medical centers [17].

Rates of carbapenem resistance among *P. aeruginosa* isolates in 6 European countries were more than 25%, among which Greece had the highest rate (51%) [1].

Imipenem and meropenem are regularly prescribed for the treatment of nosocomial infections caused by MDR *P. aeruginosa*, but increased resistance to these antibiotics have restricted their efficiency [16, 23].

The prevalence of imipenem resistance among *P. aeruginosa* isolates of Saudi Arabia and Egypt was about 39% [1]. A similar high rate of resistance has been reported in many developing countries throughout the world [24]. Recently, the rate of imipenem resistance in other regions of our country, such as Tehran, Ahvaz, and Hamedan has been reported to be from 11% to 61% [16].

High levels of resistance to antimicrobial agents were observed in the present research. The rate of resistance to imipenem, meropenem and ceftazidime in our *P. aeruginosa* isolates were, respectively, 97.9%, 97.9%, and 79.2%, which showed higher resistance in comparison with other studies.

This increased rate of carbapenem resistance reveals a threat, restricting medical care options in our hospitals. It can be explained partially by increased consumption of broad-spectrum antibiotics, which has caused a selective pressure of antibiotics on *P. aeruginosa* and consequently alteration of resistance mechanisms in the bacteria. Some studies reported emergence of colistin resistant organisms [17]. Our findings showed that 48.5% of isolates were resistant to colisitin and a proper selection of antibiotics should be made to treat such patients.

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Therefore, administration of empirical anti-pseudomonal antibiotics, including the aminoglycosides, ticarcillin, ceftazidime, cefepime, aztreonam, the carbapenems, ciprofloxacin, and levofloxacin should be considered in patients with nosocomial infections caused by MDR *P. aeruginosa* strains [2].

Due to these different results, every institution should routinely monitor resistance rate of pathogens and antibiotics consumption, and unify the information into infection control programs [17].

Also, screening of carbapenemase producers among carbapenem-resistant *P. aeruginosa* isolates is required [19].

Phenotypic tests, such as MHT can be performed as a primary screening test to reduce the costs [18]. Carbapenemase genes were detected only in colonies, because of possible false-positive results of EDTA due to altered OprD levels; also, PCR confirmation is a gold standard [6, 12]. Fisher exact test showed a statistically significant difference between the presence of bla_{IMP} gene and MDR positivity (p=0.009).

In our study, all of the 22 MHT positive cases for *VIM* and *IMP* were confirmed positive by PCR technique.

MBLs have been reported from many regions, from which it can be suggested that these enzymes are an important mechanism of carbapenem resistance in *P. aeruginosa*. MBLs belong to Ambler class B carbapenemases, and *AIM* (Adelaide imipenemase), *GIM*, *IMP*, *NDM*, *SPM*, and *VIM* types of MBLs have been reported worldwide [17]. Up to now different families of these enzymes have been reported from several countries, among which IMP- and VIM-producing *Pseudomonas* strains have been reported globally [25].

In our study, 44.9% of total 48 *P. aeruginosa* isolates were MBL producer, which was higher than the prevalence of MBL producers in Egyptian studies (27% and 32.3%) and Indian study (28.57%) [1].

However, the rapid spread of MBLs, especially among *P. aeruginosa* strains, is an emerging threat, causing nosocomial infections [20]. MBLs of the *IMP*, *VIM*, and *SIM* families have been mostly detected in imipenem-resistant *P. aeruginosa* strains in Iran [23].

The *VIM* enzymes are also most frequent in Korea (88%) and Greece (85%) [26]. Previous studies indicated that *IMP* and *VIM* types of MBLs are also prevalent in Asian countries [27-29]. *VIM* type is also highly widespread in Turkey [30]. In previous studies in Egypt (2014) and Taiwan (2013), VIM-2 showed the highest prevalence among imipenem-resistant *P. aeruginosa* strains [1, 17].

In previous similar studies in Iran (Tehran and Sanandaj), most of the MBL-producing *P. aeruginosa* isolates were bla_{VIM} positive and no isolate was positive for bla_{IMP} gene by PCR [27]. A majority of these isolates were resistant to all ß-lactam antibiotics, except monobactams [16]. In another previous study performed on burn patients in Ahvaz, 19.51% of isolates had bla_{VIM} genes, but no isolate was found positive for bla_{IMP} genes [31]. Our findings showed that 31.3% of isolates were bla_{IMP} positive and 14.6% were bla_{VIM} positive. This different prevalence of MBL-producing *P. aeruginosa* in Iranian studies, may be because of differences in geographic locations [23].

In this study, all isolates were negative for bla_{SME}, bla_{AIM}, bla_{SPM}, bla_{GIM}, or bla_{NDM} genes by multiplex-PCR screening, which is in accordance with another similar study in sanandaj, Iran [23].

During the last decades, several types of MBL enzymes have been identified in *P. aeruginosa* in different countries, such as IMP-1 that is widespread in Japan and China and is emerging in Europe and Canada [26]. There are few reports of NDM-1 in *P. aeruginosa* strains, but because of their ability to live in different environmental conditions and possibility of their spread in hospital settings, cautious vigilance and constant surveillance of these strains have been recommended [1]. Emergence of MBL-producing strains in health care settings can cause serious global nosocomial infections as well as problems in infection control management [16, 30, 31].

Our study has some limitations. Firstly, this was a retrospective study, and we were not able to investigate several independent factors. Secondly, although we detected the presence of bla_{VIM} and bla_{IMP} genes, we could not detect *AIM*, *SME*, *GIM*, *SPM*, *NDM* genes, probably because of our small sample size. Further studies are recommended to clarify if the presence of carbapenem-resistance genes can affect the clinical outcome.

Given that patients with infections caused by MBL-producing strains are at an increased risk of treatment failure, rapid detection of these organisms is recommended. The multiplex PCR test could complement phenotypic tests. The prevalence of *VIM* and *IMP* in the present study was relatively high and these MBL enzymes could emerge among hospitalized patients.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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