View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Qazvin University of Medical Sciences Repository

Iranian Journal of Microbiology
Volume 7 Number 6 (December 2015) 302-309

ORIGINAL ARTICLE

Dissemination of *Pseudomonas aeruginosa* producing bla_{IMP-1} and bla_{VIM-1} in Qazvin and Alborz educational hospitals, Iran

Amir Peymani, Taghi Naserpour Farivar, Mahdi Mohammadi Ghanbarlou, Reza Najafipour*

Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

Received: July 2015, **Accepted:** October 2015

ABSTRACT

Background and Objectives: *Pseudomonas aeruginosa* is a frequent opportunistic pathogen in health care associated infections that is highly resistant to the majority of β-lactams. The aims of this study were to access the antimicrobial susceptibility pattern of *P. aeruginosa* isolated from educational hospitals of Qazvin and Alborz provinces, to determine the prevalence of metallo-β-lactamase (MBL) among carbapenem non-susceptible isolates by combined disk (CD) method, and to detect the bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{GIM}, bla_{SPM} and bla_{NDM-1}-MBL genes.

Materials and Methods: In this cross-sectional study, 300 *P. aeruginosa* isolates were collected from different clinical specimens in two provinces of Qazvin and Alborz hospitals, Iran. After identification of isolates by standard laboratory methods, antimicrobial susceptibility was done against 17 antibiotics according to clinical and laboratory standards institute (CLSI) guideline. CD method was carried out for detection of MBLs and the presence of bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{GIM}, bla_{NDM-1} and bla_{SPM}-genes was further assessed by PCR and sequencing methods.

Results: In this study, 107 (35.66%) isolates were non-susceptible to imipenem and/or meropenem among those 56 (52.3%) isolates were metallo-β-lactamase producer. Twenty-four of 56 (42.85%) MBL-positive isolates were confirmed to be positive for MBL-encoding genes in which 14 (25%) and 10 (17.85%) isolates carried bla_{IMP-1} and bla_{VIM-1} genes either alone or in combination. Three (5.35%) isolates carried bla_{IMP} and bla_{VIM} genes, simultaneously.

Conclusion: Considering the moderate prevalence and clinical importance of MBL-producing isolates, rapid identification and use of appropriate infection control (IC) measures are necessary to prevent further spread of infections by these resistant organisms.

Keywords: *Pseudomonas aeruginosa,* Antibiotic resistance, Metallo-β-lactamase

INTRODUCTION

Pseudomonas aeruginosa is one of the most prevalent opportunistic human pathogen causing several clinical infections including wound infection, pneumonia, urinary tract infections, endocarditis, meningitis, brain abscess, and bacteremia (1-3). The increasing inappropriate use of broad-spectrum antibiotics has increased the appearance of multidrug resistant *P. aeruginosa* (MDRPA) isolates which complicates the process of therapy and limits treatment options (4).

Multidrug resistant is defined as being resistant to at least 3 anti-pseudomonal antibiotic-groups including β-lactams, aminoglycosides, and fluoroquinolones (5). Carbapenems are antibiotics used for treatment of hospitalized patients infected with MDRPA (6). These antibiotics are a class of β-Lactam antibiotics with a broad spectrum of antibacterial activity and have the broadest antibacterial spectrum compared to other β-lactams such as penicillins and cephalosporins (7). However, the incidence

*Corresponding author: Dr. Reza Najafipour
Address: Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran.
E-mail: rnajafipour@gmail.com

of carbapenem resistance among clinical isolates of *P. aeruginosa* has been raised, predominantly due to production of carbapenemases, including the imipenem metallo-β-lactamase (IMP), VIM (Verona imipenemase), SPM (São Paolo metallo-β- lactamase), and GIM (German imipenemase) (3, 8).

MBLs were first formally categorized from serine β-lactamases in 1980 in molecular classification scheme proposed by Ambler (9). In 1989, Bush further classified MBLs into a separate group (group 3) according to their functional properties (10). The MBLs-mediated resistance is important emerging resistance mechanisms in *P. aeruginosa* and is therefore associated with significant morbidity and mortality (3, 11). MBL activity is inhibited by metal chelators, such as EDTA and THIOL compounds. These enzymes can hydrolyze most beta-lactam antibiotics including, penicillins, cephalosporins and carbapenems except monobactams (12, 13).

The bla_{IMP}, bla_{VIM}, bla_{SPM} and bla_{NDM-1}-encoding genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria (6). The first reported of mobile MBLs was with the discovery of *P. aeruginosa* strain GN17203 from Japan in 1988 (14). The IMP enzymes were originally detected in Asia, but later spread to Europe, to the United States and to Australia, while the VIM gene was first found in Europe, and shortly after emerged to other countries (3). The aims of this study were (i) to access the antimicrobial susceptibility of *P. aeruginosa* isolated from educational hospitals of Qazvin and Alborz provinces, (ii) to determine the prevalence of MBLs among carbapenem non-susceptible isolates by CD test, (iii) and to detect the bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{GIM}, bla_{SPM} and bla_{NDM-1} genes in MBL-producing isolates.

MATERIALS AND METHODS

Study design and identification. A total of 300 non-repetitive *P. aeruginosa* isolates were obtained from the different clinical specimens of patients admitted in Qazvin and Alborz educational hospitals. The bacterial isolates were collected from January of 2011 until November 2013. These isolates were identified by standard laboratory methods including bacteriologic and biochemical methods such as; Gram staining, oxidase test, growth at 42°C, growth on cetrimide agar medium (Liofilchem, Italy), O/F (Oxidation-Fermentation) test and pigment production (15). The isolates were collected from various clinical specimens including tracheal aspirate, urine, sputum, blood, wound, and bronco alveolar lavage. These isolates were stored at -70°C in trypticase soy broth containing 20% glycerol and sub cultured twice prior to testing.

Antimicrobial susceptibility testing. This test was performed using Kirby-Bauer disc diffusion method according to the CLSI guideline (16). The following antibiotic discs were used: cefepime (30 µg), amikacin (30 µg), aztreonam (30 µg), polymyxin B (10 µg), imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), piperacillin/tazobactam (100/10µg), piperacillin (100 µg), ceftazidim (30 µg), cefotaxime (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ofloxacin (10 µg), ticarcillin (10 µg), carbenicillin (10 µg) and tobramycin (10 µg). Antibiotic discs were purchased from Mast (Mast Diagnostics Group Ltd, Merseyside, UK). *P. aeruginosa* ATCC 27853 was used as the quality control strain in antimicrobial susceptibility testing.

MBL screening and confirmation by phenotypic methods. Kirby-Bauer disk diffusion test was performed in order to screen MBL production using imipenem (10µg) and meropenem (10 µg) disks. Isolates that were non-susceptible to carbapenem antibiotics in combined disc method, were used as a potential MBL producer. In brief a 0.5 M EDTA solution was prepared and then was added to 10 µg imipenem disks to obtain a concentration of 750 µg. The bacterial suspension equivalent to 0.5 McFarland turbidity was prepared and was inoculated onto plates of Mueller–Hinton agar as recommended by the CLSI. The imipenem and imipenem-EDTA disks were placed on the plate. The inhibition zones of these disks with and without EDTA solution were compared after 16–18 hours of incubation in air at 35ºC. An increase of ≥7 in the zone diameter for imipenem in the presence of EDTA was considered as positive result (13).

Detection of MBL- encoding genes by PCR and sequencing. All MBL-producing isolates were subjected to PCR for detection of bla_{IMP-1}, bla_{IMP-2}, bla_{VIM-1}, bla_{VIM-2}, bla_{SPM}, bla_{SIM}, bla_{GIM} and bla_{NDM-1} genes, using specific primers (Table 1) (17-19).

Table 1. Oligonucleotide primers used in this study

Genes	Sequence (5'→3')	Reference
bla_{IMP-1} F	ACCGCAGCAGAGTCTTTGCC	17
bla_{IMP-1} R	ACAACCAGTTTTGCCTTACC	
bla_{IMP-2} F	GTTTTATGTGTATGCTTCC	17
bla_{IMP-2} R	AGCCTGTTCCCATGTAC	
bla_{VIM-1} F	AGTGGTGAGTATCCGACAG	17
bla_{VIM-1} R	ATGAAAGTGCGTGGAGAC	
bla_{VIM-2} F	ATGTTCAAACTTTTGAGTAAG	17
bla_{VIM-2} R	CTACTCAACGACTGAGCG	
bla_{SPM-1} F	GCGTTTTGTTTGTTGCTC	17
bla_{SPM-1} R	TTGGGGATG TGAGACTAC	
bla_{GIM} F	TCGACACACCTTGGTCTG	18
bla_{GIM} R	AACTTCCAACTTTGCCAT	
bla_{SIM} F	TACAAGGGATTCGGCATCC	18
bla_{SIM} R	TAATGGCCTGTTCCCATG	
bla_{NDM-1} F	GGCGGAATGGCTCATCACGA	19
bla_{NDM-1} R	CGCAACACAGCCTGACTTTC	

Total DNAs were extracted by the DNA extraction kit (Bioneer Company-Korea). PCR amplifications were performed in a thermocycler (Applied Biosystems, USA) as follows: 95ºC for 5min and 35 cycles of 1min at 95ºC, 1min at specific annealing temperature for each primer and 1min at 72ºC. A final extension step of 10 min at 72ºC was performed. Amplification reactions were prepared in a total volume of 25µl (24µl of PCR master mix plus 1µl of template DNA) including 5ng of genomic DNA, 2.0U of Taq DNA polymerase, 10mM dNTP mix at a final concentration of 0.2mM, 50mM $MgCl_2$ at a final concentration of 1.5mM, 1µM of each primer, and 1X PCR buffer (final concentration). PCR products were electrophoresed on 1% agarose gel and then were stained with ethidium bromide solution and finally visualized using gel documentation system (UVtec, UK). The purified PCR products were sequenced by the Macrogen Company (Seoul, South Korea) and the sequence alignment and analysis were performed online using the BLAST program of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

Statistical analysis. Statistical data analysis was performed for descriptive statistics, including frequencies, cross tabulation of microbiological, clinical features and demographic characteristics using the computer software program SPSS version 16.

RESULTS

In this study, 96 (32 %) isolates were collected from the patients admitted to intensive care unit (ICU), 100 (33.3 %) infection diseases, 61 (20.3 %) internal medicine, 30 (10 %) surgery, 8 (2.7 %) neurosurgery and 5 (1.7 %) neurology wards. Our bacterial isolates were recovered from blood (108-36%), urine (91-30.33%), wound (34-11.33%), bronco alveolar lavage (23-7.66 %), trachea secretions (23-7.66%) and sputum (21-7%) samples. One hundred fifty-six patients (52%) were female and 144 (48%) were male. The mean age of the patients was 51.07±18.8 (range 18–90) years.

Antimicrobial susceptibility. The high rates of antibiotic resistance were against cefotaxime (92.7 %) and aztreonam (57.7%). Polymyxin B, amikacin and piperacillin/tazobactam revealed high susceptibility rates of 98.3%, 71.3% and 64.7 %, respectively. One hundred one (33.7%) isolates were characterized as MDRPA i.e., were intermediate or resistance to at least three different classes of antimicrobial agents including β-lactams, aminoglycosides and fluoroquinolones. In total, 107 (35.66%) isolates were

non-susceptible to imipenem and/or meropenem (Table 2).

Detection of MBL by CD and PCR methods. Among 107 carbapenem non-susceptible *P. aeruginosa* isolates, 56 (52.3%) isolates were MBL producer. PCR and sequencing showed that 24 (42.85%) isolates were positive for MBL genotypes among those 14 (25%) isolates and 10 (17.85%) isolates carried bla_{IMP-1} and bla_{VIM-1} genes either alone or in combination. Three (5.35%) isolates carried both the bla_{IMP-1} and bla_{VIM-1} genes, simultaneously. Isolates were negative for bla_{IMP-2}, bla_{VIM-2}, bla_{GIM}, bla_{SIM}, bla_{NDM-1} and bla_{SPM} genes (Table3).

Table 2. Antimicrobial susceptibility of *P. aeruginosa* isolated from hospitals of Qazvin and Alborz provinces

	Number (%)		
Antibiotics	**Resistant**	**Intermediate**	**Susceptible**
Cefotaxime	(7%.52)158	(40%)120	(3%.7)22
Aztreonam	(36%) 115	(21.7%) 65	(40%) 120
Carbenicillin	(7%.51)155	(7%.4)14	(7%.43)131
Levofloxacin	(7%.48)146	(2%)6	(3%.49)148
Ofloxacin	(47%)141	(7%.3)11	(3%.49)148
Ticarcillin	(3%.47)142	(3%.2) 7	(3%.50)151
Ciprofloxacin	(3%.43)130	(7%.3)11	(53%)159
Gentamicin	(7%.44)134	(3%.1)4	(54%)162
Tobramycin	(7%.42)128	(7%.1)5	(7%.55)167
Ceftazidime	(37%)111	(3%.5)16	(7%.57)173
Piperacillin	(3%.32)97	(7%.9)29	(58%)174
Meropenem	(35%)105	(3%.5)16	(7%.59)179
Imipenem	(31%)93	(7%)21	(62%)186
Cefepime	(33%) 99	(4%)12	(63%) 189
Piperacillin/tazobactam	(7%.25)77	(9.7%)29	(64.7%)194
Amikacin	(38.3%) 62	(8%)24	(71.3%) 214
Polymyxin B	(1.7%)5	0	(3%.98)295

Table 3. Case histories and characteristics of the 24 bla_{IMP} and bla_{VIM}-producing *P. aeruginosa* isolates collected from Qazvin and Alborz hospitals

Isolates	Age (yr)/ gender	Ward	Source	Antibiotic susceptibility profile	MBL genes
P.A 16	71/F	ICU	Blood	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= S, ATM=R, LEV=R, PRL=R,CPD= R	bla_{VIM-1}
P.A 26	32/M	Internal	Blood	IMP= R,MEM= R, CPM= R, AMK= S, CTX= I, CAZ= S, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= S, ATM=I, LEV=R, PRL=R,CPD= R	bla_{VIM-1}
P.A 76	62/F	Infection	Urine	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= I, OFX= R, TN= R, ATM=I, LEV=R, PRL=I,CPD= R	bla_{VIM-1}
P.A 77	47/M	Infection	Wound	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= R, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{VIM-1}
P.A 79	49/F	Infection	Urine	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{VIM-1}
P.A 92	29/F	ICU	BAL	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{IMP-1}

Table 3. Countinued ...

P.A 113	30/M	Infection	Urine	IMP= R,MEM= I, CPM= S, AMK= S, CTX= R, CAZ= S, TC= R, CIP= S, GM=R, PB= S, PTZ= S, OFX= R, TN= R, ATM=S, LEV=R, PRL=S,CPD= R	bla_{VIM-1}
P.A 117	24/F	Infection	Blood	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= S, ATM=R, LEV=R, PRL=R,CPD= R	bla_{IMP-1} + bla_{VIM-1}
P.A 123	50/F	Surgery	Wound	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{IMP-1}
P.A 124	47/M	Infection	Urine	IMP= R,MEM= R, CPM= R, AMK= S, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= I, OFX= R, TN= R, ATM=I, LEV=R, PRL=R,CPD= R	bla_{IMP-1}
P.A 159	33/F	Infection	Blood	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{IMP-1}
P.A 179	57/F	Internal	Urine	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{IMP-1} + bla_{VIM-1}
P.A 183	46/F	ICU	BAL	IMP= R,MEM= R, CPM= R, AMK= I, CTX= R, CAZ= R, TC= S, CIP= R, GM=R, PB= S, PTZ= S, OFX= S, TN= R, ATM=R, LEV=S, PRL=S,CPD= R	bla_{IMP-1}
P.A 196	24/F	Infection	Blood	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{IMP-1}
P.A 198	36/F	Internal	Blood	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{IMP-1}
P.A 207	42/F	ICU	Urine	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{IMP-1}
P.A 212	33/F	Internal	Blood	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= I, OFX= R, TN= S, ATM=I, LEV=R, PRL=R,CPD= R	bla_{IMP-1}
P.A 224	61/M	Infection	Urine	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=S,CPD= R	bla_{IMP-1} + bla_{VIM-1}
P.A 241	39/M	Surgery	Blood	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= I, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= S, ATM=R, LEV=R, PRL=R,CPD= R	bla_{VIM-1}
P.A 242	59/F	ICU	Blood	IMP= R,MEM= R, CPM= R, AMK= I, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=I,CPD= R	bla_{IMP-1}
P.A 278	61/M	Infection	Urine	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= S, OFX= S, TN= S, ATM=S, LEV=S, PRL=S,CPD= R	bla_{IMP-1}
P.A 283	74/F	ICU	Blood	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= R, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{IMP-1}
P.A 292	37/F	Internal	Urine	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= I, OFX= R, TN= R, ATM=R, LEV=R, PRL=I,CPD= R	bla_{IMP-1}
P.A 295	77/M	Surgery	Wound	IMP= R,MEM= R, CPM= R, AMK= R, CTX= R, CAZ= R, TC= R, CIP= R, GM=R, PB= S, PTZ= I, OFX= R, TN= R, ATM=R, LEV=R, PRL=R,CPD= R	bla_{IMP-1}

R: Resistance; I: Intermediate; S: Susceptible; ATM: Aztreonam; CTX: Cefotaxime; CAZ: Ceftazidime; PIP: Piperacillin; TZP: Piperacillin-tazobactam; CPM: Cefepime; GEN: Gentamicin; CIP: Ciprofloxacin; LEV: Levofloxacin; AMK: Amikacin; MEM: Meropenem; IPM: Imipenem; PB: Polymyxin B; TN: Tobramycin; OFX: Ofloxacin; TC: Ticarcillin; PY: Carbenicillin

DISCUSSION

Despite the rapid development of antibiotics and improvement in supportive care, *P. aeruginosa* remains one of the most serious health care associated infections with high rate of mortality and morbidity. This organism exhibits high rates of resistance to the several classes of antimicrobial agents (20). Carbapenems (imipenem and meropenem) are the most active agents for treating of nosocomial infections caused by *P. aeruginosa* isolates (3). However, the emergence of carbapenem resistant *P. aeruginosa* isolates has be-

come a serious clinical concern because of its intrinsic and acquired resistance mechanisms, limiting the treatment options (21). MBLs are increasingly widespread around worldwide as important mechanisms of carbapenem resistance among *P. aeruginosa* (3, 6, 22).

In the present study, antimicrobial susceptibility results showed that 33.7% isolates were MDRPA with significant rate of resistance against common used antibiotics. These findings are in agreement with results of previous studies in Iran, which reported that *P. aeruginosa* isolates are resistant to many classes of antibiotics (23, 24).

Overall, resistance to imipenem and/or meropenem was 35.66% which is higher than results of other studies have been reported by Shahcheraghi et al. (12.4%) and Japoni et al. (30%) in Iran (24, 25), but lower than that found in two previous studies carried out by Sepehriseresht et al. (56%) and Khosravi (41%) et al. in burn patients in Iran (26, 27). The varied range in susceptibility rate of carbapenems among *P. aeruginosa* isolates in different studies could be because of varied antibiotic usage profiles in different geographic regions. These results indicate that the available choices for the appropriate treatment of infection caused by MDRPA are currently limited.

In this study, 52.3% of carbapenem non-susceptible isolates were MBL producers which are higher than those reported by Khosravi et al. from Iran (19.5%) (27), Nagaveni et al. from India (20.8%) (28), Ellington et al. from UK (38.3%) (29) and Pitout et al. from Germany (46%) (30), indicating the MBL-producing *P. aeruginosa* is increasing. It should be noted that 48.7% of carbapenem resistant *P. aeruginosa* were MBL negative which suggest other mechanisms might alternatively be contributed in the carbapenem resistance, most importantly production of oxacilinase or deficiency in porin or reduced expression of outer membrane proteins (3). We previously reported that 31 (49%) of *A. baumannii* isolates were found to be MBL producers (31).

In the present study, of 56 MBL-producing isolates, 24 (42.85%) isolates were positive for MBL genotypes which 14 (25%) isolates and 10 (17.85%) isolates carried $bla_{\text{IMP-1}}$ and $bla_{\text{VIM-1}}$ genes either alone or in combination. In recent years, bla_{IMP} and bla_{VIM}-producing *P. aeruginosa* isolates have been reported in Asian countries. In a study from Iran, Sarhangi et al. reported that 8 (9.75%) and 10 (12.19%) of MBL-producing *P. aeruginosa* isolates were positive for $bla_{\text{IMP-1}}$ and $bla_{\text{VIM-1}}$ genes, respectively (32). In another study from Iran, Khosravi et al. reported that 19.51% of clinical isolates of *P. aeruginosa* contained bla_{VIM} gene (27). In South Korea, Oh et al. reported that 82.85% and 5.71% of *P. aeruginosa* isolates harbored $bla_{\text{VIM-2}}$ and $bla_{\text{IMP-1}}$ genes, respectively (33). In another study from South Korea, Ryoo et al. reported that 15.6% of *P. aeruginosa* isolates harbored $bla_{\text{IMP-1}}$ and 8.6% of isolates harbored $bla_{\text{VIM-2}}$ (34). In Turkey, Ozqumus et al. reported that 9% and 1% of *P. aeruginosa* isolates were positive for $bla_{\text{IMP-1}}$ and bla_{VIM} genes, respectively (35). In France, Pitout et al. showed that 43% and 2% of *P. aeruginosa* isolates carried bla_{VIM} and bla_{IMP} genes, respectively (30).

This study showed that most $bla_{\text{IMP-1}}$ and $bla_{\text{VIM-1}}$-positive *P. aeruginosa* isolates were frequently collected from the patients admitted to intensive care and infection disease units. It seems that chronic underlying conditions, prolonged period of ICU stay and the use of invasive techniques and devices predispose patients to infection with these resistant isolates.

In conclusion, results of this study revealed the considerable prevalence of MBL-producing *P. aeruginosa* isolates in our hospital settings. Moreover, the MBL-encoding genes often carried by mobile genetic elements which can rapidly spread horizontally between different strains. However, early recognition of MBL-producing isolates and establishing tactful policies associated with infection control measures and appropriate antimicrobial therapy based on laboratory data are necessary to reduce further spread of these resistant bacteria in our hospitals.

ACKNOWLEDGMENT

This study was financially supported by the Cellular and Molecular Research Center and Research Deputy of Qazvin University of Medical Sciences, Qazvin, Iran.

REFERENCES

1. Sako S, Kariyama R, Mitsuhata R, Yamamoto M, Wada K, Ishii A, et al. Molecular epidemiology and clinical implications of metallo-β-lactamase-producing *Pseudomonas aeruginosa* isolated from urine. *Acta Med Okayama* 2014;68: 89-99.
2. Bisbe J, Gatell JM, Puig J, Mallolas J, Martinez JA, Jimenez de Anta MT, et al. *Pseudomonas aeruginosa*

bacteremia: univariate and multivariate analyses of factors influencing the prognosis in 133 episodes. *Rev Infect Dis* 1988;10: 629-635.
3. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β-lactamases, the quiet before the storm? *Clin Microbiol Rev* 2005;18: 306-25.
4. Bratu S, Quale J, Cebular S, Heddurshetti R, Landman D. Multidrug resistant *Pseudomonas aeruginosa* in Brooklyn, New York: molecular epidemiology and in vitro activity of polymyxin B. *Eur J Clin Microbiol Infect Dis* 2005;24: 196-201.
5. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, 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: 268-281
6. Nordmann P, Poirel L. Emerging carbapenemases in gram-negative aerobes. *Clin Microbial Infect* 2002;8: 321-31.
7. Lagatolla C, Tonin EA, Monti-Bragadin C, Dolzani L, Gombac F, Bearzi C, et al. Endemic carbapenem-resistant *Pseudomonas aeruginosa* with acquired metallo-beta-lactamase determinants in European hospital. *Emerg Infect Dis* 2004;10: 535-8.
8. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev* 2007;20: 440-458.
9. Ambler RP. The structure of β-lactamases. *Philos Trans R Soc Lond B Biol Sci* 1980;289: 321-331.
10. Bush, K. Classification of beta-lactamases: groups 2c, 2d, 2e, 3, and 4. *Antimicrob Agents Chemother* 1989;33: 271-6.
11. Peleg AY, Franklin C, Bell JM, Spelmann DW. Dissemination of the metallo-β-lactamase gene bla IMP4 among gram-negative pathogens in a clinical setting in Australia. *Clin Infect Dis* 2005;41: 1549-56.
12. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-β-lactamase-producing gram-negative bacteria by using thiol compounds. *J Clin Microbiol* 2000;38: 40-43.
13. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2002;40: 3798-3801.
14. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in *P. aeruginosa*. *Antimicrob Agents Chemother* 1991;35: 147 -51.
15. Mahon CR, Lehman DC, Manuselis G. Textbook of diagnostic microbiology. 4th ed. Maryland Heights, Mo.: Saunders/Elsevier; 2011.
16. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: seventeenth informational supplement M100-S23. USA: Wayne, PA; 2013.
17. Shibata N, Doi Y, Yamane K, Yagi T, Kurokawa H, Shibayama K, et al. Y. PCR typing of genetic determinants for metallo-β-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. *J Clin Microbiol* 2003; 41: 5407-13.
18. Jovcic B, Lepsanovic Z, Suljagic V, Rackov G, Begovic J, Topisirovic L, et al. Emergence of NDM-1 metallo-β-lactamase in *Pseudomonas aeruginosa* clinical isolates from Serbia. *Antimicrob Agents Chemother* 2011; 55: 3929-31.
19. Chen Y, Zhou Z, Jiang Y, Yu Y. Emergence of NDM-1-producing *Acinetobacter baumannii* in China. *J Antimicrob Chemother* 2011; 66: 1255-9.
20. Jones RN, Kirby JT, Beach ML, Biedenbach DJ, Pfaller MA. Geographic variations in activity of broad spectrum β-lactams against *Pseudomonas aeruginosa*: summary of the worldwide SENTRY antimicrobial surveillance program (1997–2000). *Diagn Microbiol Infect Dis* 2002;43: 239-243.
21. Saderi H, Lotfalipour H, Owlia P, Salimi H. Detection of metallo-β-Lactamase producing *Pseudomonas aeruginosa* isolated from burn patients in Tehran, Iran. *Lab Medicine* 2010;41: 609-12.
22. Hancock RE. Resistance mechanisms in *Pseudomonas aeruginosa* and other non-fermentative gram negative bacteria. *Clin Infect Dis* 1998;27: S93-9.
23. Tavajjohi Z, Moniri R. Detection of ESBLs and MDR in *Pseudomonas aeruginosa* in a tertiary-care teaching hospital. *Arch Clin Infect Dis* 2011;6: 18-23.
24. Japoni A, Alborzi A, Kalani M, Nasiri J, Hayati M, Farshad SH. Susceptibility patterns and cross-resistance of antibiotics against *Pseudomonas aeruginosa* isolated from burn patients in the south of Iran. *Burns* 2006;32: 343-347.
25. Shahcheraghi F, Nikbin VS, Feizabadi MM. Identification and genetic characterization of metallo-β-lactamase -producing strains of *Pseudomonas aeruginosa* in Tehran, Iran. *New Microbiol* 2010;33: 243-248.
26. Sepehriseresht S, Boroumand MA, Pourgholi L, Sotoudeh Anvari M, Habibi E, Sattarzadeh Tabrizi M. Detection of vim- and ipm-type metallo-β-lactamases in *Pseudomonas aeruginosa* clinical isolates. *Arch Iran Med* 2012;15: 670-673.
27. Khosravi AD, Mihani F. Detection of metallo betalactamase- producing *Pseudomonas aeruginosa* strains isolated from burn patients in Ahwaz, Iran. *Diagn Microbiol Infect Dis* 2008;60: 125-8.
28. Nagaveni S, Rajeshwari H, Oli AK, Patil SA, Chandrakanth RK. Widespread emergence of multidrug resistant *Pseudomonas aeruginosa* isolated from CSF samples. *Indian J Microbiol* 2011;51: 2-7.

29. Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. *J Antimicrob Chemother* 2007;59: 321-322.
30. Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL. Detection of *Pseudomonas aeruginosa* producing metallo-β-lactamase s in a large centralized laboratory. *J Clin Microbiol* 2005;43: 3129-35.
31. Peymani A, Nahaei MR, Farajnia S, Hasani A, Mirsalehian A, Sohrabi N, et al. High prevalence of metallo-β-lactamase -producing *Acinetobacter baumannii* in a teaching hospital in Tabriz, Iran. *Jpn J Infect Dis* 2011;64: 69-71.
32. Sarhangi M, Motamedifar M, Sarvari J. Dissemination of *Pseudomonas aeruginosa* Producing bla_{IMP1}, bla_{VIM2}, bla_{SIM1}, bla_{SPM1} in Shiraz, Iran. *Jundishapur J Microbiol* 2013;6: e6920.
33. Oh EJ, Lee S, Park YJ, Park JJ, Park K, Kim SI, et al. Prevalence of metallo-beta-lactamase among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in a Korean university hospital and comparison of screening methods for detecting metallo-β-lactamase. *J Microbiol Methods* 2003;54:411-418.
34. Ryoo NH, Ha JS, Jeon DS, Kim JR. Prevalence of Metallo-β-lactamases in Imipenem-non-susceptible *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Korean J Clin Microbiol* 2010;13: 169-172.
35. Ozgumus OB, Caylan R, Tosun I, Sandalli C, Aydin K, Koksal I. Molecular epidemiology of clinical *Pseudomonas aeruginosa* isolates carrying IMP-1 metallo-β-lactamase gene in a University Hospital in Turkey. *Microb Drug Resist* 2007;13: 191-198.