Dissemination of *Pseudomonas aeruginosa* producing *bla*<sub>IMP-1</sub> and *bla*<sub>VIM-1</sub> in Qazvin and Alborz educational hospitals, Iran

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**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*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>SIM</sub>, *bla*<sub>GIM</sub>, *bla*<sub>NDM</sub> and *bla*<sub>SPM</sub>-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*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>SIM</sub>, *bla*<sub>GIM</sub>, *bla*<sub>NDM</sub> and *bla*<sub>SPM</sub>-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*<sub>IMP-1</sub> and *bla*<sub>VIM-1</sub> genes either alone or in combination. Three (5.35%) isolates carried *bla*<sub>IMP</sub> and *bla*<sub>VIM</sub> 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
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 β-lactam antibiotics including, penicillins, cephalosporins and carbapenems except monobactams (12, 13).

The *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>SPM</sub> and *bla*<sub>NDM-1</sub>-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*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>SMT</sub>, *bla*<sub>GAM</sub>, *bla*<sub>SPM</sub> and *bla*<sub>NDM-1</sub> 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), carbencillin (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 35°C. An increase of ≥7 in the zone diameter for imipenem (10µg) and meropenem (10 µg) disks 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*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>SMT</sub>, *bla*<sub>GAM</sub>, *bla*<sub>SPM</sub> and *bla*<sub>NDM-1</sub> genes, using specific primers (Table 1) (17-19).
Total DNAs were extracted by the DNA extraction kit (Bioneer Company-Korea). PCR amplifications were performed in a thermocycler (Applied Biosystems, USA) as follows: 95°C for 5 min and 35 cycles of 1 min at 95°C, 1 min at specific annealing temperature for each primer and 1 min at 72°C. A final extension step of 10 min at 72°C was performed. Amplification reactions were prepared in a total volume of 25 μl (24 μl of PCR master mix plus 1 μl of template DNA) including 5 ng of genomic DNA, 2.0 U of Taq DNA polymerase, 10 mM dNTP mix at a final concentration of 0.2 mM, 50 mM MgCl2 at a final concentration of 1.5 mM, 1 μM 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%), broncho 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

### Table 1. Oligonucleotide primers used in this study

| Genes   | Sequence (5'→3') | Reference |
|---------|-----------------|-----------|
| bla\_IMP\_1 F | ACCGCAGCAGAGTCTTTGCC | 17 |
| bla\_IMP\_2 R | AACAACATTTTGGCCTAC | 17 |
| bla\_IMP\_2 F | GTTTTATGTGTAGCTTCC | 17 |
| bla\_VIM\_1 R | AGGTGTGAGTATCGAGAC | 17 |
| bla\_VIM\_2 F | ATGGATCGTTGAGAC | 17 |
| bla\_VIM\_2 R | ATGTCCAACTTTTGGAGAAG | 17 |
| bla\_VIM\_1 F | CTACTCAACGACTGACGC | 17 |
| bla\_NDM\_1 R | GCCGTGTTTGGTGCTG | 17 |
| bla\_NDM\_1 F | TTGGGATGTCAGACTAC | 17 |
| bla\_NDM\_2 F | TCGACACCACTGGTCTG | 18 |
| bla\_NDM\_2 R | AACTTACACATTGGCCAT | 18 |
| bla\_NDM\_3 F | TACAAAGGATCAGCACCC | 18 |
| bla\_NDM\_3 R | TAATGGCCTGTTCCCATG | 18 |
| bla\_NDM\_4 F | GCGGGAATGGCTCACTCAGA | 19 |
| bla\_NDM\_4 R | CGCAACACAGCCTGACTTC | 19 |
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*<sub>IMP-1</sub> and *bla*<sub>VIM-1</sub> genes either alone or in combination. Three (5.35%) isolates were negative for *bla*<sub>IMP-2</sub>, *bla*<sub>VIM-2</sub>, *bla*<sub>GIM</sub>, *bla*<sub>SIM</sub>, *bla*<sub>NDM-1</sub> and *bla*<sub>SPM</sub> genes (Table 3).

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

| 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         | (7.4%)131       |
| Levofloxacin      | (7.48)146     | (2%)6            | (3.4%)148       |
| Ofloxacin         | (47%)141      | (7.3%)11         | (3.4%)148       |
| Ticarcillin       | (3.47)142     | (3%)7            | (3.5%)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        |
| Ceferpine         | (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*<sub>IMP</sub> and *bla*<sub>VIM</sub>-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*<sub>VIM-1</sub> |
| P.A 26   | 32/M     | Internal | Blood | IMP= R, MEM= R, CPM= R, AMK= S, CTX= L, 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*<sub>VIM-1</sub> |
| 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*<sub>VIM-1</sub> |
| 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*<sub>VIM-1</sub> |
| 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*<sub>VIM-1</sub> |
| 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*<sub>IMP-1</sub> |
**Table 3. Continued ...**

| P.A  | Age | Sex | Site         | Pathogen   | Antibiotics | Carbapenem Resistance |
|------|-----|-----|--------------|-------------|-------------|------------------------|
| 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 | blaIMP-1, blaVIM-1 |
| 117  | 24/F|     | Blood        | IMP R, MEM R, CPM R, AMK R, CTX R, CAZ R, TC S, CIP R, GM R, PB S, PTZ R, OFX R, TN S, ATM R, LEV R, PRL R, CPD R | blaIMP-1 |
| 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 | blaIMP-1 |
| 124  | 47/M|     | 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 R, LEV R, PRL R, CPD R | blaIMP-1 |
| 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 | blaIMP-1 |
| 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 | blaIMP-1, blaVIM-1 |
| 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 | blaIMP-1 |
| 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 | blaIMP-1 |
| 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 | blaIMP-1 |
| 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 | blaIMP-1 |
| 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 | blaIMP-1 |
| 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 | blaIMP-1, blaVIM-1 |
| 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 | blaIMP-1 |
| 242  | 59/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 I, CPD R | blaIMP-1 |
| 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 | blaIMP-1 |
| 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 | blaIMP-1 |
| 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 | blaIMP-1 |
| 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 | blaIMP-1 |

R: Resistance; I: Intermediate; S: Susceptible; ATM: Aztreonam; CTX: Cefotaxime; CAZ: Ceftazidine; 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 the world 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 rates 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 Shahrcheraghi 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 oxacillinase 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 blaIMP-1 and blaVIM-1 genes either alone or in combination. In recent years, blaIMP and blaVIM-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 blaIMP-1 and blaVIM-1 genes, respectively (32). In another study from Iran, Khosravi et al. reported that 19.51% of clinical isolates of P. aeruginosa contained blaVIM-1 gene (27). In South Korea, Oh et al. reported that 82.85% and 5.71% of P. aeruginosa isolates harbored blaVIM-2 and blaIMP-1 genes, respectively (33). In another study from South Korea, Ryoo et al. reported that 15.6% of P. aeruginosa isolates harbored blaIMP-1 and 8.6% of isolates harbored blaVIM-2 (34). In Turkey, Ozqumus et al. reported that 9% and 1% of P. aeruginosa isolates were positive for blaIMP-1 and blaVIM genes, respectively (35). In France, Pitout et al. showed that 43% and 2% of P. aeruginosa isolates carried blaVIM and blaIMP genes, respectively (30).

This study showed that most blaIMP-1 and blaVIM-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.

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