

# Detection and Genetic Characterization of Metallo- $\beta$ -Lactamase *IMP-1* and *VIM-2* in *Pseudomonas aeruginosa* Strains From Different Hospitals in Kermanshah, Iran

Ramin Abiri<sup>1,\*</sup>; Pantea Mohammadi<sup>2</sup>; Navid Shavani<sup>1</sup>; Mansour Rezaei<sup>3</sup>

<sup>1</sup>Department of Microbiology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, IR Iran

<sup>2</sup>Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, IR Iran

<sup>3</sup>Department of Biostatistics and Epidemiology, School of Hygiene, Kermanshah University of Medical Sciences, Kermanshah, IR Iran

\*Corresponding author: Ramin Abiri, Department of Microbiology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, IR Iran. Tel: +98-9122773648, Fax: +98-8314276471, E-mail: rabiri@kums.ac.ir

Received: August 6, 2014; Revised: November 26, 2014; Accepted: December 19, 2014

**Background:** *Pseudomonas aeruginosa* is a frequent nosocomial pathogen that causes severe diseases in many settings. Carbapenems, including meropenem and imipenem, are effective antibiotics against this organism. However, the use of carbapenems has been hampered by the emergence of strains resistant to carbapenems via different mechanisms such as the production of metallo- $\beta$ -lactamases (MBLs), which hydrolyze all carbapenems. Several kinds of MBLs have been reported, among them *VIM* and *IMP* types being the most clinically significant carbapenemases.

**Objectives:** We aimed to determine the distribution of *bla*<sub>VIM-2</sub> and *bla*<sub>IMP-1</sub> transferable genes encoding MBLs in *P. aeruginosa* isolated from three academic hospitals in Kermanshah.

**Patients and Methods:** From 22nd June to 22nd September 2012, 225 isolates of *P. aeruginosa* were collected. These isolates were tested for antibiotic susceptibility with the Kirby-Bauer disk-diffusion method, and the MBLs were assessed using the imipenem-EDTA double-disk synergy test. The isolates were investigated for *bla*<sub>VIM-2</sub> and *bla*<sub>IMP-1</sub> genes using polymerase chain reaction.

**Results:** Among the 225 isolates, 33.7% (76/225) and 18.1% (41/225) were resistant to imipenem and meropenem, respectively. Of the 76 imipenem-resistant *P. aeruginosa* strains, 45 (59.2%) were positive for MBLs, 34 (75%) strains carried the *bla*<sub>IMP-1</sub> gene, and 1 (2.2%) strain carried the *bla*<sub>VIM-2</sub> gene.

**Conclusions:** Our results showed that there was a high frequency of *IMP-1* positive *P. aeruginosa* in the different wards of the hospitals.

**Keywords:**  $\beta$ -Lactamases; Carbapenems; Polymerase Chain Reaction; *Pseudomonas aeruginosa*

## 1. Background

*Pseudomonas aeruginosa* is a frequent nosocomial pathogen that causes severe diseases in many settings, particularly in immune-compromised patients such as those with cancer, burns, and cystic fibrosis. Infections are frequently severe, and two recent studies have indicated that the rate of mortality attributable to *P. aeruginosa* bacteremia is approximately 34% (1, 2). This organism is clinically important since it possesses several virulence factors and is intrinsically resistant to many antimicrobial and disinfectant agents (3). Carbapenems such as meropenem and imipenem are effective antibiotics against this organism since they present a good spectrum of activity and are stable to hydrolysis by most  $\beta$ -lactamases, including the extended spectrum  $\beta$ -lactamases (3, 4). However, the use of carbapenems has been hampered by the emergence of strains resistant to carbapenems via different mechanisms such as impermeability to the drug due to loss of OprD porin, upregulation of the active efflux pump systems, and production of metallo- $\beta$ -lactamases (MBLs), which hydrolyze all carbapenems (5-7).

MBLs are divided into two categories: chromosomally mediated enzymes and those encoded by movable genetic elements such as plasmids and transposons (8-10). Several kinds of MBLs have been reported, including *IMP*, *VIM*, *SPM*, *GIM*, and *SIM-1* (11). The *VIM* and *IMP* types are the most clinically significant carbapenemases coded by *bla*<sub>VIM</sub> and *bla*<sub>IMP</sub> genes. At least 14 different *VIM* and 23 different *IMP* MBLs have been identified so far (7, 8). There are many reports which indicate an increase in the prevalence of carbapenem-resistance mediated by acquired MBLs; however, the prevalence of the MBL-producing isolates of *P. aeruginosa* varies in Asian countries (8, 12, 13). In Iran, the first isolation of *bla*<sub>IMP-1</sub> was reported in 2011 by Peymani et al. (14), who collected *Acinetobacter baumannii* isolates from several hospitals in Tabriz (Northwest of Iran).

The growing rate of resistance against the traditional antibiotics has rendered the treatment of patients with infections caused by MBL-producing *P. aeruginosa* critical. Previous studies have shown a high prevalence rate of multidrug-resistant *P. aeruginosa* isolated from differ-

ent clinical specimens, especially in burn wards in Iran (15,16); nevertheless, little information is available on the distribution of MBL-producing isolates in the country. For example, in Markazi province, 37% of the *P. aeruginosa* isolates obtained from the hospitalized patients were shown to be resistant to imipenem, and 50% of these strains were detected to have *bla*<sub>VIM-1</sub>, 56.6% *bla*<sub>VIM-2</sub>, and 6.6% *bla*<sub>IMP-1</sub> (17). In another study, from 240 *P. aeruginosa* isolates, 34.16% were imipenem-resistant, and 23.3% were screened as MBL-positive strains using the double-disk synergy test. Additionally, a specific polymerase chain reaction (PCR) test confirmed the presence of 8 (9.75%) and 10 (12.19%) *IMP-1* and *VIM-2* types, respectively (18).

## 2. Objectives

The current study was performed to examine the prevalence of MBL-producing *P. aeruginosa* isolates obtained from clinical specimens at different hospitals in Kermanshah (Imam Khomeini, Imam Reza, and Taleghani hospitals) and also the prevalence of those harboring the *bla*<sub>VIM-2</sub> and *bla*<sub>IMP-1</sub> genes, which are the most clinically significant carbapenemases.

## 3. Patients and Methods

### 3.1. Samples

From 22nd June to 22nd September 2012, 225 isolates of *P. aeruginosa* were collected from three hospitals in Kermanshah. The isolates were taken from urine (n = 79, 35%), blood (n = 14, 6.2%), wound (n = 29, 12.8%), respiratory tract (n = 81, 36%), burned tissue (n = 17, 7.5%), and eye (n = 5, 2.2%). The isolates were collected from different wards, including ICU, CCU, urology, emergency, burn, respiratory, and surgery. *P. aeruginosa* was identified by Gram staining, ability to produce oxidase and catalase, oxidative-fermentative test, resistance to ceftriaxone, and growth at 42°C (19).

### 3.2. Antimicrobial Susceptibility Testing

The disk diffusion test was conducted in accordance with the clinical and laboratory standards institute (CLSI) methodology. The antimicrobial agents included comprised aztreonam (10 µg), cefepime (10 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg),

and piperacillin/tazobactam (10 µg, 100 µg) (MAST, U.K.). *P. aeruginosa* ATCC 27853 was used as quality control in each run of antimicrobial susceptibility testing.

### 3.3. Identification of Metallo-β-Lactamases

Among β-lactamases, MBLs are unique in requiring the presence of zinc ion in the active site of the enzyme, and are, thus, inhibited by chelating agents such as ethylenediaminetetraacetic acid (EDTA) (20). The imipenem-EDTA double-disk synergy test was used in order to identify the *P. aeruginosa* producing MBLs. Therefore, a 0.50-M EDTA (Merck, Germany) solution was prepared by dissolving 186.1 g of EDTA in 1000 mL of distilled water, and the pH was adjusted to 8 by adding NaOH (21). Then, 4 µL of the prepared solution was added to the imipenem disk and dried in an incubator. The prepared EDTA/imipenem disk and the imipenem disk itself were placed in a plate containing the Müller-Hinton agar (Merck, Germany) with cultured *P. aeruginosa*. After 16 - 18 hours of incubation at 37°C, an increase of ≥ 7 mm in the inhibition zone diameter in the presence of EDTA compared to imipenem tested alone was considered to be a positive test for the presence of an MBL (4, 18, 22).

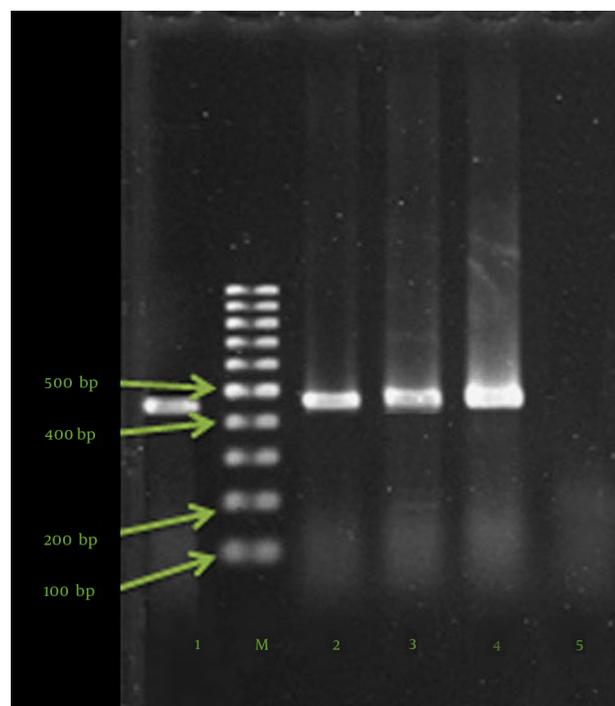
PCR was performed to detect the *bla*<sub>VIM-2</sub> and *bla*<sub>IMP-1</sub> genes. The DNA templates from all the isolates were extracted by boiling (10 minutes in 95°C), and PCR was carried out in a thermal cycler (Bio Rad, USA) using 25-µL volumes containing 12.5 µL of PCR Master Mix (500 mM of Tris-HCl pH 8.55, 1.5 mM of MgCl<sub>2</sub>, 0.2 mM of dNTPs, and 0.04 units/µL of Taq DNA polymerase) (SinaClon Inc., Tehran, Iran) and 0.5 µM of each of the primers (SinaClon Inc., Iran). The primers and the cycling condition are listed in Table 1 (23, 24).

Following amplification, 10 µL of each sample was analyzed with 1.5% agarose gel electrophoresis for the detection of positive samples. For all the amplification reactions, the mixture was heated at 96°C for 4 minutes prior to thermocycling. The mixture was held at 72°C for 7 minutes after the final cycle before cooling at 4°C. The agarose gel electrophoresis of *bla*<sub>VIM-2</sub> and *bla*<sub>IMP-1</sub> PCR products is depicted in Figure 1. The positive controls (clinical *P. aeruginosa* isolates with sequenced *bla*<sub>IMP-1</sub> and *bla*<sub>VIM-2</sub> genes) were kindly dedicated by Dr. Najjar Pirayeh (Tarbiat Modarres University), and the negative control contained water in place of DNA.

**Table 1.** Primers and Cycling Condition

Target	Oligonucleotide Sequence (5' - 3')	Polymerase Chain Reaction Condition	No. of Cycles	Fragment Size, bp	Reference
<i>bla</i> <sub>IMP-1</sub>		94°C, 30 s	25	448	(23)
	CATGGTTTGGTGGTCTCTGT	57°C, 45 s			
	ATAATTTGGCGGACTTTGGC	72°C, 60 s			
<i>bla</i> <sub>VIM-2</sub>		94°C, 30 s	25	801	(24)
	ATGTTCAAACITTTGAGTAAG	57°C, 45 s			
	CTACTCAACGACTGAGCG	72°C, 60 s			

**Figure 1.** Electrophoresis Results of the *bla*<sub>IMP-1</sub> Gene Polymerase Chain Reaction



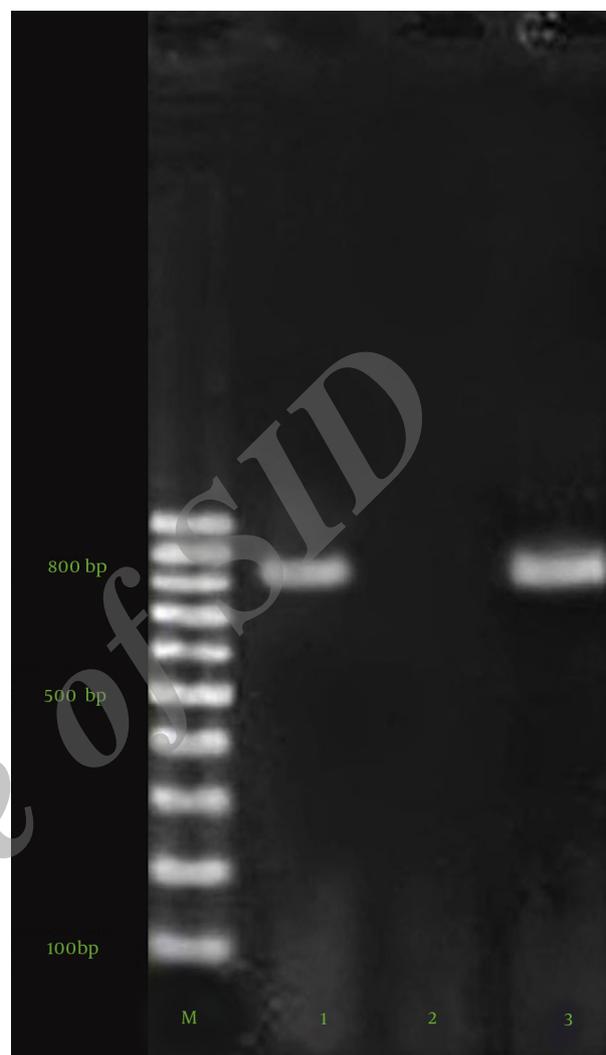
Lane 1: Positive control (clinical *P. aeruginosa* with the sequenced *bla*<sub>IMP-1</sub> gene). Lane M: 100 bp DNA ladder. Lanes 2, 3, and 4: Isolates with the *bla*<sub>IMP-1</sub> gene. Lane 5: Negative control (distilled water).

#### 4. Results

Totally, 225 confirmed isolates of *P. aeruginosa* recovered from different clinical specimens were tested for antibiogram. The findings showed that 137 (61%) isolates were resistant to ceftazidime, 131 (58.5%) to gentamicin, 130 (57.7%) to piperacillin/tazobactam, 101 (44.7%) to ciprofloxacin, 96 (42.8%) to cefepime, 92 (40.9%) to aztreonam, 76 (33.7%) to imipenem, and 41 (18.1%) to meropenem. According to these percentages, imipenem and meropenem were the most effective drugs. The imipenem-EDTA double-disk synergy test showed that 45 out of the 76 (59.2%) imipenem-resistant strains were MBL positive. The isolates were collected from different wards, including ICU (n = 22), urology (n = 2), emergency (n = 4), burn (n = 11), respiratory (n = 3), and surgery (n = 3).

Furthermore, our molecular studies showed that only the MBL-positive strains were positive in PCR. Moreover, 75% (n = 34) of the MBL-positive strains were detected to have *bla*<sub>IMP-1</sub> and 2.2% *bla*<sub>VIM-2</sub> (n = 1). *bla*<sub>IMP-1</sub> was taken from burned tissue (n = 10, 29.4%), respiratory tract (n = 10, 29.4%), urine (n = 8, 23.5%), blood (n = 2, 5.8%), eye (n = 3, 8.8%), and vagina (n = 1, 2.9%). *bla*<sub>VIM-2</sub> (n = 1) was recovered from burned tissue. Antibiogram showed that the strains resistant to imipenem possessed high-level resistance to gentamicin (100%), cefepime (94.7%), and ceftazidime

**Figure 2.** Electrophoresis Results of the *bla*<sub>VIM-2</sub> Gene Polymerase Chain Reaction



Lane M: 100 bp DNA ladder. Lane 1: Isolate with the *bla*<sub>VIM-2</sub> gene. Lane 2: Negative control (distilled water). Lane 3: Positive control (clinical *P. aeruginosa* with the sequenced *bla*<sub>VIM-2</sub> gene).

(94.7%). Among the imipenem-resistant strains, 30.3% (n = 23) were resistant to meropenem and the rest (n = 53) were sensitive to it. On the other hand, there were 18 meropenem-resistant strains sensitive to both imipenem and cefepime.

#### 5. Discussion

Microbial resistance has increased drastically in recent years in both developed and developing countries and it has rapidly become a leading public health concern. The prevalence of antimicrobial resistance varies greatly between and within countries and between different pathogens. Resistance to antibiotics among organisms is increased either by mutations or by acquiring new genetic material via horizontal gene transfer. Several factors

favor the development of bacterial resistance to antibiotics in developing countries such as the less potent activity of the drugs, i.e. some of the antibiotics provided in developing countries have decreased potency due to the degradation or adulteration of the drug or because of the presence of a lower concentration of active substances, lack of well-equipped diagnostic laboratories, and excessive use of antimicrobials in domestic animals (8-10).

Antimicrobial agents suitable for use in pediatric clinics are mainly limited to  $\beta$ -lactam antibiotics, especially carbapenems. However, the problem of drug resistance to carbapenems is gradually worsening owing to their increasing clinical use. The present study analyzed the patterns of resistance to several antibiotics such as carbapenems. The results showed that 33.7% of the isolates were resistant to imipenem. In a previous study in the Iranian city of Kermanshah in 2004, Mohajeri et al. (25) reported a lower rate of resistance (10%). However, another investigation conducted in the Iranian city of Shiraz in 2012 reported that 127 (59.16%) of the 240 isolates were resistant to this antibiotic (18). The reported rates of resistance to imipenem in Japan, Russia, France, Canada, and Spain were 8.3%, 13.4%, 18.5%, 12%, and 14%, respectively (26-30). As was mentioned, numerous factors contribute to these variations. In addition, the increased rate of resistance to imipenem in Kermanshah is in line with the increase throughout the world. Resistance to carbapenems in *P. aeruginosa* may be due to MBLs, which hydrolyze all carbapenems (5-7). Since MBLs are placed in movable elements, they are horizontally transmitted between organisms easily (8-10, 31). Large outbreaks by MBL-producing *P. aeruginosa* strains have been described in hospitals in Italy, Greece, and Korea (32-37). *IMP* and *VIM*-producing *P. aeruginosa* strains have been reported worldwide (10, 38-42).

In our study, the majority of the MBL producers carried the *bla<sub>IMP</sub>* gene (75%); however, the presence of this gene has been previously proved to be variable in different regions (17, 18). In a previous report from the Iranian province of Markazi, 37% (40 out of 108) of the *P. aeruginosa* isolates obtained from the hospitalized patients were demonstrated to be resistant to imipenem. The EDTA/imipenem test showed that 50% of the imipenem-resistant strains were MBL positive, and PCR revealed that 50% of the imipenem-resistant strains had *bla<sub>VIM-1</sub>*, 56.6% *bla<sub>VIM-2</sub>*, and 6.6% *bla<sub>IMP-1</sub>* (17). In another study in Iran, from 240 *P. aeruginosa* isolates mainly recovered from wound, urine, and sputum, 82 (34.16%) isolates were imipenem-resistant (minimum inhibitory concentration [MIC]  $\geq 4$   $\mu$ g/mL). Among these imipenem-resistant isolates, 19 (23.3%) MBL-producing *P. aeruginosa* isolates were screened using the double-disk synergy test. A specific PCR test confirmed the presence of 8 (9.75%) and 10 (12.19%) *IMP-1* and *VIM-2* types, respectively (18). In contrast, Khosravi and Mihani (2008) reported that none of the 8 MBL-producing *P. aeruginosa* strains in their study were positive for the *bla<sub>IMP</sub>* gene (40).

The most and also the sole frequent reported MBL-pro-

ducing *P. aeruginosa* in Iran is *VIM-1*, previously cited by several researchers in different cities of Iran (4, 17, 22, 40, 43, 44). Nonetheless, there are only a few investigations reporting the presence of the *VIM-2* type of *P. aeruginosa* in Iran. For the first time in 2012, Sadeghi et al. (17) detected this genotype in the Iranian city of Arak. Subsequently, in 2013, among the 82 imipenem-resistant isolates tested for MBLs, 10 isolates were positive for this gene (18). According to our findings, it seems that the MBL-producing isolates of *P. aeruginosa* are the main cause of IPM resistance among this species. We suggest that molecular typing via standard methods such as pulsed-field gel electrophoresis be conducted to define the clonality of the isolates.

## Acknowledgements

This study was conducted in the microbiology department of Kermanshah university of medical sciences. The authors are grateful to Dr. Najjar Pirayeh for providing the positive controls.

## Authors' Contributions

Ramin Abiri: study concept and design, interpretation of data and final revision of the manuscript; Pantea Mohammadi: research and technical advisor, drafting of the manuscript; Navid Shavani: contribution in all laboratory experiments; Mansour Rezaei: Statistical analysis.

## Funding/Support

The financial costs of the study were provided by Kermanshah university of medical sciences.

## References

- Giamarellou H. Prescribing guidelines for severe Pseudomonas infections. *J Antimicrob Chemother.* 2002;**49**(2):229-33.
- Rello J, Jubert P, Valles J, Artigas A, Rue M, Niederman MS. Evaluation of outcome for intubated patients with pneumonia due to Pseudomonas aeruginosa. *Clin Infect Dis.* 1996;**23**(5):973-8.
- Gales AC, Menezes LC, Silbert S, Sader HS. Dissemination in distinct Brazilian regions of an epidemic carbapenem-resistant Pseudomonas aeruginosa producing SPM metallo-beta-lactamase. *J Antimicrob Chemother.* 2003;**52**(4):699-702.
- Shahcheraghi F, Nikbin VS, Feizabadi MM. Identification and genetic characterization of metallo-beta-lactamase-producing strains of Pseudomonas aeruginosa in Tehran, Iran. *New Microbiol.* 2010;**33**(3):243-8.
- Kohler T, Michea-Hamzehpour M, Epp SF, Pechere JC. Carbapenem activities against Pseudomonas aeruginosa: respective contributions of OprD and efflux systems. *Antimicrob Agents Chemother.* 1999;**43**(2):424-7.
- Livermore DM. Interplay of impermeability and chromosomal beta-lactamase activity in imipenem-resistant Pseudomonas aeruginosa. *Antimicrob Agents Chemother.* 1992;**36**(9):2046-8.
- Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. *Clin Microbiol Infect.* 2002;**8**(6):321-31.
- Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? *Clin Microbiol Rev.* 2005;**18**(2):306-25.
- Pitout JD, Revathi G, Chow BL, Kabera B, Kariuki S, Nordmann P, et al. Metallo-beta-lactamase-producing Pseudomonas aeruginosa isolated from a large tertiary centre in Kenya. *Clin Microbiol Infect.* 2008;**14**(8):755-9.

10. Garza-Ramos U, Morfin-Otero R, Sader HS, Jones RN, Hernandez E, Rodriguez-Noriega E, et al. Metallo-beta-lactamase gene bla(IMP-15) in a class 1 integron, In95, from *Pseudomonas aeruginosa* clinical isolates from a hospital in Mexico. *Antimicrob Agents Chemother*. 2008;**52**(8):2943-6.
11. Mendes RE, Castanheira M, Pignatari ACC, Gales AC. Metallo-beta-lactamases. *Jornal Brasileiro de Patologia e Medicina Laboratorial*. 2006;**42**(2):103-13.
12. Iseri L, Bayraktar MR. Changes in the rates of antimicrobial resistance among clinical isolates of *Pseudomonas aeruginosa* between 2002 and 2004 in a tertiary-care teaching hospital in Turkey. *New Microbiol*. 2008;**31**(3):351-5.
13. Picao RC, Poirel L, Gales AC, Nordmann P. Diversity of beta-lactamases produced by ceftazidime-resistant *Pseudomonas aeruginosa* isolates causing bloodstream infections in Brazil. *Antimicrob Agents Chemother*. 2009;**53**(9):3908-13.
14. Peymani A, Nahaei MR, Farajnia S, Hasani A, Mirsalehian A, Sohrabi N, et al. High prevalence of metallo-beta-lactamase-producing acinetobacter baumannii in a teaching hospital in Tabriz, Iran. *Jpn J Infect Dis*. 2011;**64**(1):69-71.
15. Shahcheraghi F, Feizabadi MM, Yamin V, Abiri R, Abedian Z. Serovar determination, drug resistance patterns and plasmid profiles of *Pseudomonas aeruginosa* isolated from burn patients at two hospitals of Tehran (IRAN). *Burns*. 2003;**29**(6):547-51.
16. Shahcheraghi F, Nikbin VS, Feizabadi MM. Prevalence of ESBLs genes among multidrug-resistant isolates of *Pseudomonas aeruginosa* isolated from patients in Tehran. *Microb Drug Resist*. 2009;**15**(1):37-9.
17. Sadeghi A, Rahimi B, Shojapour M. Molecular detection of metallo-β-lactamase genes blaVIM-1, blaVIM-2, blaIMP-1, blaIMP-2 and blaSPM-1 in *Pseudomonas aeruginosa* isolated from hospitalized patients in Markazi province by Duplex-PCR. *Afr J Microbiol Res*. 2012;**6**(12):2965-9.
18. Sarhangi M, Motamedifar M, Sarvari J. Dissemination of *Pseudomonas aeruginosa* Producing blaIMP1, blaVIM2, blaSIM1, blaSPM1 in Shiraz, Iran. *Jundishapur J Microbiol*. 2013;**6**(7):e6920.
19. Mahon C, Lehman D, Manuvelis G. *Text book of Diagnostic Microbiology*. 4 ed. Maryland Heights (MO): Saunders Elsevier; 2011.
20. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother*. 1995;**39**(6):1211-33.
21. Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning*. New York: Cold spring harbor laboratory press; 1989.
22. Shahcheraghi F, Nikbin VS, Shooraj F, Shafiei M. Investigation of blaIMP-1, blaVIM-1 and blaSPM-1 MBL genes among clinical strains of *Pseudomonas aeruginosa* isolated from Imam Khomeini Hospital, Tehran, Iran. *Pejouhandeh*. 2009;**14**(2):Pe67-72.
23. Fazeli H, Sadighian H, Esfahani BN, Pourmand MR. Identification of class-1 integron and various β-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* at children's medical center hospital. *J Med Bacteriol*. 2013;**1**(3, 4):25-36.
24. Shibata N, Doi Y, Yamane K, Yagi T, Kurokawa H, Shibayama K, et al. PCR typing of genetic determinants for metallo-beta-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. *J Clin Microbiol*. 2003;**41**(12):5407-13.
25. Mohajeri P. Antibiotic susceptibility and resistance patterns of *pseudomonas aeruginosa* strains isolated from different clinical specimens in patients referred to the teaching hospitals in Kermanshah (2001-2). *J Kermanshah Univ Med Sci*. 2004;**7**(4):11-20.
26. Bouza E, Garcia-Garrote F, Cercenado E, Marin M, Diaz MS. *Pseudomonas aeruginosa*: a survey of resistance in 136 hospitals in Spain. The Spanish *Pseudomonas aeruginosa* Study Group. *Antimicrob Agents Chemother*. 1999;**43**(4):981-2.
27. Rio Y, Pina P, Jurin F, Allouch P, Didion J, Chardon H, et al. [Susceptibility of *Pseudomonas aeruginosa* to antibiotics isolated from patients of intensive care units in France in 1998. Resistant phenotypes to beta-lactams]. *Pathol Biol (Paris)*. 2002;**50**(1):12-7.
28. Karakoc B, Gerceker AA. In-vitro activities of various antibiotics, alone and in combination with amikacin against *Pseudomonas aeruginosa*. *Int J Antimicrob Agents*. 2001;**18**(6):567-70.
29. Sivoldskii EP. [Antibiotics sensitivity and characteristics of the esculin-positive *Pseudomonas aeruginosa* biovar]. *Antibiot Khimioter*. 2000;**45**(8):17-20.
30. Niitsuma K, Saitoh M, Kojimabara M, Kashiwabara N, Aoki T, Tomizawa M, et al. [Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated in Fukushima Prefecture]. *Jpn J Antibiot*. 2001;**54**(2):79-87.
31. 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**(4):169.
32. Hsueh PR, Teng LJ, Yang PC, Chen YC, Ho SW, Luh KT. Persistence of a multidrug-resistant *Pseudomonas aeruginosa* clone in an intensive care burn unit. *J Clin Microbiol*. 1998;**36**(5):1347-51.
33. Cornaglia G, Riccio ML, Mazzariol A, Lauretti L, Fontana R, Rossolini GM. Appearance of IMP-1 metallo-beta-lactamase in Europe. *Lancet*. 1999;**353**(9156):899-900.
34. Hirakata Y, Yamaguchi T, Nakano M, Izumikawa K, Mine M, Aoki S, et al. Clinical and bacteriological characteristics of IMP-type metallo-beta-lactamase-producing *Pseudomonas aeruginosa*. *Clin Infect Dis*. 2003;**37**(1):26-32.
35. Lee K, Lim JB, Yum JH, Yong D, Chong Y, Kim JM, et al. bla(VIM-2) cassette-containing novel integrons in metallo-beta-lactamase-producing *Pseudomonas aeruginosa* and *Pseudomonas putida* isolates disseminated in a Korean hospital. *Antimicrob Agents Chemother*. 2002;**46**(4):1053-8.
36. Mavroidi A, Tsakris A, Tzelepi E, Pournaras S, Loukova V, Tzouveleki LS. Carbapenem-hydrolysing VIM-2 metallo- beta-lactamase in *Pseudomonas aeruginosa* from Greece. *J Antimicrob Chemother*. 2000;**46**(6):1041-2.
37. Senda K, Arakawa Y, Nakashima K, Ito H, Ichiyama S, Shimokata K, et al. Multifocal outbreaks of metallo-β-lactamase-producing *Pseudomonas aeruginosa* resistant to broad-spectrum -lactams, including carbapenems. *Antimicrob Agents Chemother*. 1996;**40**:349-53.
38. Dong F, Xu XW, Song WQ, Lu P, Yu SJ, Yang YH, et al. Characterization of multidrug-resistant and metallo-beta-lactamase-producing *Pseudomonas aeruginosa* isolates from a paediatric clinic in China. *Chin Med J (Engl)*. 2008;**121**(17):1611-6.
39. Yatsuyanagi J, Saito S, Harata S, Suzuki N, Ito Y, Amano K, et al. Class 1 integron containing metallo-beta-lactamase gene blaVIM-2 in *Pseudomonas aeruginosa* clinical strains isolated in Japan. *Antimicrob Agents Chemother*. 2004;**48**(2):626-8.
40. Khosravi AD, Mihani F. Detection of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* strains isolated from burn patients in Ahwaz, Iran. *Diagn Microbiol Infect Dis*. 2008;**60**(1):125-8.
41. Corvec S, Poirel L, Espaze E, Giraudeau C, Drugeon H, Nordmann P. Long-term evolution of a nosocomial outbreak of *Pseudomonas aeruginosa* producing VIM-2 metallo-enzyme. *J Hosp Infect*. 2008;**68**(1):73-82.
42. Perez IA, Garcia CP, Poggi MH, Braun JS, Castillo VC, Roman JC, et al. [Presence of metallo beta-lactamases in imipenem-resistant *Pseudomonas aeruginosa*]. *Rev Med Chil*. 2008;**136**(4):423-32.
43. Saderi H, Lotfalipour H, Owlia P, Salimi H. Detection of Metallo-β-lactamase Producing *Pseudomonas aeruginosa* Isolated From Burn Patients in Tehran, Iran. *Lab Med*. 2010;**41**(10):609-12.
44. Bahar MA, Jamali S, Samadikuchaksaraei A. Imipenem-resistant *Pseudomonas aeruginosa* strains carry metallo-beta-lactamase gene bla(VIM) in a level I Iranian burn hospital. *Burns*. 2010;**36**(6):826-30.