



The Study of Frequency of *SIM* and *AmpC* Genes in Clinical Isolates of *Pseudomonas aeruginosa* in Gilan, Iran

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ABSTRACT

Aims: This study aimed to determine the antibiotic resistance pattern of *Pseudomonas aeruginosa* clinical isolates and the frequency of *blaSIM* and *blaAmpC* genes in resistant strains. **Materials & Methods:** In this cross-sectional study, 94 *P. aeruginosa* isolates were collected from the burn wards of Gilan province hospitals in 2018 and identified by biochemical methods. Strains producing β -lactamases and metallo- β -lactamases were detected by two methods: disk diffusion method and antibiotic resistance method in combination with disk diffusion method, respectively. The presence of *blaSIM* and *blaAmpC* genes in the resistant strains was investigated using PCR, and data analysis was performed.

Findings: Based on the obtained results, colistin was identified as the most effective antibiotic with a resistance rate of 27.7%, and the highest antibiotic resistance was observed against trimethoprim/sulfamethoxazole (83%). In the phenotypic test of 94 samples, 29 (30.9%) carbapenemase-producing isolates and 33 (35.1%) β -lactamase-producing isolates were identified. Based on the PCR results, among 44 (46.8%) samples containing β -lactamase and carbapenemase enzymes, the frequency of *blaSIM* gene was 9.1% (4 of 44, and 4.3% in all the studied isolates), and the frequency of *blaAmpC* gene was 15.9% (7 of 44, and 7.4% in all the studied isolates).

Conclusion: The results of this study indicated a high prevalence of drug resistance in clinical isolates of *P. aeruginosa*. In particular, there was an increasing rate of resistance to beta-lactam antibiotics, and the presence of MBL and ESBL associated genes was considerable, which limit the choice of suitable treatment for patients with severe infections.

Keywords: *Pseudomonas aeruginosa*, Antibiotic resistance, PCR, *blaSIM*, *blaAmpC*.

CITATION LINKS

- [1] Arabestani MR, Rajabpour M, Yousefi Mashouf R, Alikhani MY, Mousavi SM. Expression of ... [2] Rajabpour M, Alikhani MY. MIC determination of *Pseudomonas* ... [3] Wong YP, Chua KH, Thong KL. One-step species-specific high-resolution melting analysis for ... [4] Brooks GF, Carroll KC, Butel JS, Jawetz, Melnick & Adelberge's Medical ... [5] Akpaka PE, Legal B, Padman J. Molecular detection and epidemiology of and extended-spectrum beta-lactamase genes prevalent in clinical isolates of *Klebsiella pneumoniae* and *E.coli* from Trinidad and Tobago. West Indian ... [6] Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents ... [7] Shahcheraghi F, Shakibaie MR, Noveiri H. Molecular ... [8] Walsh TR, Bolmstrom A, Qvarnstrom A, Gales A. Evaluation of a new Etest for detection metallo-beta-lactamases in ... [9] Poirel L, Guibert M, Girlich D, Naas T, Nordmann P. Cloning, sequence analyses, expression, and distribution of *ampC-ampR* from *Morganella morganii* clinical isolates. Antimicrob Agents ... [10] Pitout JD, Chow BL, Gregson DB, Laupland KB, Elsayed S, Church DL. Molecular epidemiology of ... [11] Hall GS. Bailey & Scott's Diagnostic ... [12] Altaai ME, Aziz IH, Marhoon AA. Identification *Pseudomonas* .. [13] Khosravi AD, Moloudsadat Motahar M, Abbasi Montazeri E [14] Cayci YT, Coban AY, Gunaydin M. Investigation of ... [15] Fazeli N, Momtaz H. Virulence gene profiles of multidrug-resistant ... [16] Oh EJ, Lee S, Park YJ, Park JJ, Park K, Kim SI, et al. Prevalence of ... [17] Gendrin C, Contreras-Martel C, Bouillot S, Elsen S, Lemaire D, Skoufias DA, et al. Structural ... [18] Imani Foolad A, Rostami Z, Shapouri R. Antimicrobial resistance and ... [19] Sadeghi A, Rahimi B, Shojapour M. Molecular detection of ... [20] Taghvaei R, Shojapour M, Sadeghi A, Pourbabaie AA. The study of antibiotic ... [21] Mardaneh J, Ahmadi K, Jahan Sepas A. Determination ... [22] Rajaei S, Kazemi-Pour N, Rokhbakhsh-Zamin F. Frequency of plasmid-mediated ... [23] Sadari H, Lotfalipour H, Owlia P, Salimi H. Detection of ... [24] Radan M, Moniri R, Khorshidi A, Gilasi H, Norouzi Z, Beigi F, et al. Emerging ... [25] Nikookar I, Tishayar A, Flakiyan Z, Alijani K, Rehana-banisaeed S, Hossinpour M, et ... [26] Rafiee R, Eftekhari F, Tabatabaei SA, Minaee Tehrani D. Prevalence of ... [27] Alhaki T, Sadeghi Fard N, Saye Miri K, Bimanand L, Azizian R, Tabasi M, et al. The ... [28] Haghi F, Keramati N, Hemmati F, Zeighami H. Distribution of ... [29] Adabi J, Shahraki Zahedani S, Bokaeian M, Tahmasebi H. An investigation of ... [30] Tahmasebi H, Alikhani MY, Dehbashi S, Arabestani MR. Investigation of ... [31] Wassef M, Behiry I, Younan M, El Guindy N, Mostafa S, Abada E. Genotypic ... [32] Aksoy MD, Çavuşlu Ş, Tuğrul HM. Investigation of ...

Introduction

Pseudomonas aeruginosa is a non-fermenting Gram-negative bacillus, known as an important leading cause of infection in hospitalized patients, especially in patients with weakened immune system (including patients with burns and cystic fibrosis). Despite the improvement of health and therapeutic methods, the risk of *P. aeruginosa* infection is high in ICU patients [1]. The mortality rate of patients with *P. aeruginosa*-induced bacteremia has been reported to be 30-50%. This bacterium is an important pathogen that is more resistant to antibiotics than other Gram-negative bacteria. The intrinsic resistance of this bacterium is associated with its outer membrane with low permeability, it could also develop an acquired resistance to antibacterial agents that are typically effective on this pathogen [2-3].

Antibiotic resistance is usually based on one of the following mechanisms, and *P. aeruginosa* is not an exception: 1) the microorganism produces an enzyme that causes active drug degradation, 2) microorganisms change their permeability to the drug, 3) microorganisms change their necessary receptors for medication, and 4) microorganisms find another metabolic pathway that compensates for the drug-inhibited reaction [4]. One of the most important causes of antibiotic resistance is the production of β -lactamase enzymes [5]. The currently accepted method to classify β -lactamases is the classification based on their molecular structure and amino acid sequence. Four classes of these enzymes (A to D) and their functions have been identified. Groups A, C, and D have a serine-based function, while Group B enzymes are called metallo- β -lactamases (MBL_s) because of their need for zinc metal [6].

One of the most important factors contributing to the drug resistance of *P.*

aeruginosa to carbapenem antibiotics is the production of metallo- β -lactamase (MBL) enzymes. This has been widely studied, and different involved genes and alleles have been identified [7].

Pseudomonas carbapenemase-encoding genes are a serious clinical threat. The genes encoding these enzymes, which are located in the plasmids, could be easily transmitted to other bacteria. MBLs have a broad spectrum and are not inhibited by β -lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam; on the other hand, they could hydrolyze all β -lactam antibiotics except monobactam and aztreonam. MBL enzymes could be divided into *UIM*, *SIM*, *AIM*, *GIM*, *SPM*, or *VIM* according to their molecular structure [8-9]. *SIM* has been isolated from *Acinetobacter baumannii* strains and is a subclass of B1. *SIM* is very similar to IMP (69% to ISP12 and 64% to IMP9) and has the ability to hydrolyze a broad spectrum of β -lactams. *SIM* is located on Class I Integrons. Identifying antibiotic resistance and identifying strains harboring different *AmpC* gene families, as well as determining the prevalence and emergence of new strains, could reveal the relationship between resistance to broad-spectrum antibiotics and the presence of some of these genes [10].

Objectives: The present study was carried out to detect the frequency of *SIM* and *AmpC* genes in clinical samples of MBL-producing *P. aeruginosa* strains isolated from burn wounds of patients hospitalized in the hospitals of Gilan province.

Materials and Methods

Isolation and Identification of Bacteria:

In this descriptive cross-sectional study, conducted from August 2018 to January 2019, 94 *P. aeruginosa* clinical strains were isolated from burn wounds of patients hospitalized in the burn wards of Gilan

hospitals. The specimens were placed in plastic microtubes containing BHI transport medium (Merck, Germany) and transferred to the microbiology laboratory for complementary tests. They were cultured on MacConkey, TSI, and SIM environments (Merck, Germany). Biochemical tests including oxidase, catalase, urease, TSI, oxidation and fermentation (O/F), and growth ability of the bacteria at 42 °C were performed according to the standard detection table [11].

Determination of antibiotic resistance pattern of the isolates: Antibiotic resistance patterns of 94 *P. aeruginosa* isolates were determined by disk diffusion method according to the CLSI standard. Antibiotic discs used were as follows: ciprofloxacin (5 µg), colistin (10 µg), gentamicin (10 µg), imipenem (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), kanamycin (30 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg) (Padtan Teb).

The phenotype of β-lactamase and carbapenemase production in the isolates was ascertained by the combined disk diffusion method according to the CLSI procedure. Ceftazidime and ceftazidime + clavulanic acid were used to evaluate the strains ability to produce broad-spectrum β-lactamase, and imipenem and imipenem + EDTA discs were used to examine carbapenemase production. The standard strain of *P. aeruginosa* ATCC 27853 was used as a control. If the difference between the inhibition zone diameter of a single disc and

a disc containing an inhibitor was equal to or greater than 5 mm, that strain was identified as enzyme producer [12].

Frequency of blaSIM and AmpC genes: To identify two *blaSIM* and *AmpC* genes, the bacterial genomic DNA was extracted using the Boiling method. Briefly, bacterial isolates were cultured in LB broth medium. After reaching an appropriate turbidity, 100 µL of the culture was mixed with 400 µL of TE buffer and boiled at 95 °C for 15 min and placed at -20°C for 5 min. The vials were then centrifuged at 14000 × g for 10 min, and the supernatant was transferred from each vial to a new sterile microtube. For definitive confirmation of the extracted DNA, they were transferred onto 1% agarose gel and electrophoresed [13].

The amplification of *blaSIM* and *AmpC* genes was performed using specific primers (Table 1) and Polymerase Chain Reaction (PCR) method. PCR was performed in a total volume of 25 µL, containing 12.5 µL of PCR mixture, forward and reverse primers (10 pmol), template DNA (2 µL), and 12.5 µL sterile distilled water. The microtubes were then transferred to a thermocycler with an appropriate program described by Cayci et al. (2014) [14]. The PCR products were detected by electrophoresis on agarose gel 1%.

Findings

The antibiotic resistance pattern of 94 *P. aeruginosa* clinical isolates to 8 different antibiotics was as follows: trimethoprim/sulfamethoxazole 83%, cefotaxime 73.4%,

Table 1) Primers used for PCR detection of *SIM* and *AmpC* genes

Target Gene	Primer Sequence (5' → 3')	Product Size (bp)	Reference
<i>blaSIM-F</i> <i>blaSIM-R</i>	TAC AAG GGA TTC GGC ATC G TAA TGG CCT GTT CCC ATG TG	570	32
<i>blaAmpC-F</i> <i>blaAmpC-R</i>	CTT CCA CAC TGC TGT TCG CC TTG GCC AGG ATC ACC AGT CC	1063	26

ceftazidime 35.1%, Kanamycin 57.4%, ciprofloxacin 50%, gentamicin 45.7%, imipenem 40.4%, and colistin 27.7%. Colistin was recognized as the most potent antibiotic against clinical isolates of *P. aeruginosa*, while the highest resistance was observed to trimethoprim/sulfamethoxazole (Table 2). In phenotypic assays, 30.9 and 35.1% of the isolates were positive for metallo- β -lactamases (MBL) and extended-spectrum β -lactamases (ESBL) production, respectively. Out of 44 MBL and/or ESBL producing strains examined in PCR assay, *blaSIM* and *blaAmpC* genes were identified in 4 (4 of 44, 9.1%; 4 of 94, 4.3%) and 7 samples (4 of 44, 15.9%; 4 of 94, 7.4%), respectively (Figures 1 and 2).

Discussion

P. aeruginosa is one of the main leading causes of nosocomial infections and mortality in patients with leukemia (lymphoma), severe burns, and cystic fibrosis [15]. Due to its high drug resistance, this bacteria by itself accounts for 30% of all nosocomial infections and has made the treatment of these infections a serious problem in the health system [16].

MBLs are enzymes that confer bacterial

resistance to Imipenem [15]. The over-use of antibiotics plays a key role in the emergence of MBL-producing strains [17]. Epidemiological studies around the world have demonstrated that drug resistance pattern of *P. aeruginosa* strains could be different from country to country, from one geographical area to another, and even between different hospitals in a geographical area.

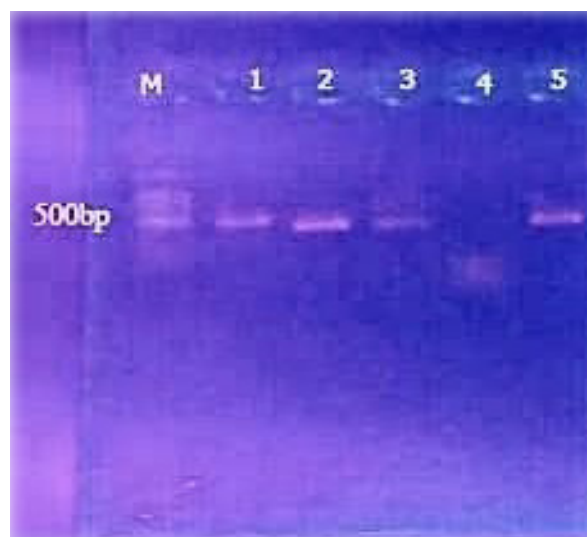


Figure 1) Electrophoresis of PCR product for *blaSIM* gene. M: 100-bp DNA marker; 4: C-; 5: C+; 1-3: the *blaSIM* gene PCR product, with an approximate length of 570 bp.

In this research, the antibiotic resistance

Table 2) Antibiotic susceptibility pattern of *P. aeruginosa* isolates.

Antibiotics	Resistant Number (%)	Intermediate Number (%)	Sensitive Number (%)
Cefotaxime	69 (73.4)	11 (11.7)	14 (14.9)
Ceftazidime	33 (35.1)	41 (43.6)	20 (21.3)
Ciprofloxacin	47 (50)	27 (28.7)	20 (21.3)
Colistin	26 (27.7)	60 (63.8)	8 (8.5)
Gentamicin	43 (45.7)	7 (7.5)	44 (46.8)
Imipenem	38 (40.4)	4 (4.3)	52 (55.3)
Kanamycin	54 (57.4)	25 (26.6)	15 (16)
Trimethoprim/ Sulfamethoxazole	78 (83)	2 (2.1)	14 (14.9)

pattern of the isolates was as follows: trimethoprim/sulfamethoxazole 83%, cefotaxime 73.4%, kanamycin 57.4%, ciprofloxacin 50%, gentamicin 45.7%, imipenem 40.4%, ceftazidime 35.1%, and colistin 27.7%.

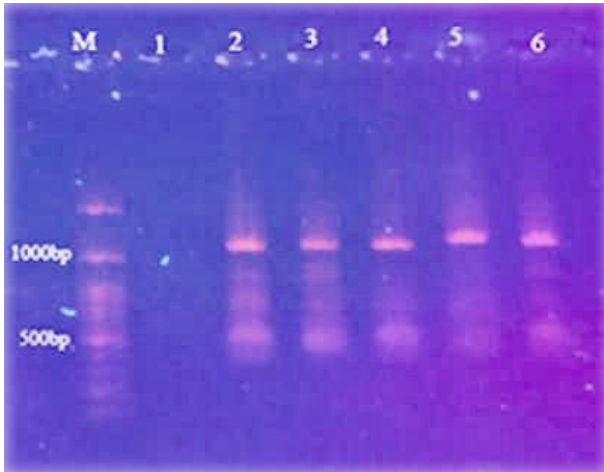


Figure 2) Electrophoresis of PCR product for *blaAmpC* gene. M: 100-bp DNA marker; 1: C-; 2: C+; 3-6: the *blaAmpC* gene PCR product with an approximate length of 1063 bp.

Imani Foolad et al. (2010) in Zanjan reported the antibiotic resistance pattern of *P. aeruginosa* isolates as follows: tetracycline (86.4%), ceftriaxone (66.4%), cefotaxime (43.6%), gentamicin (25.5%), ceftazidime (20.9%), and amikacin (17.3%)^[18]. In a study by Sadeghi et al. (2012) in Markazi province, the rate of resistance to imipenem was reported as 37%, of which 50% were MBL positive^[19]. In another research by Taghvaei et al. (2013), examining 108 *P. aeruginosa* strains collected from Arak health centers, the resistance pattern was reported as follows: ceftazidime (33.3%), imipenem (22.2%), and meropenem (24%). Out of 36 ceftazidime-resistant strains, 32 (88.8%) strains were ESBL positive^[20]. In a study in Ahvaz by Mardaneh et al. (2013), colistin exhibited the highest anti-*Pseudomonas* activity (78.3%), and there was a high resistance to beta-lactam antibacterials

^[21]. In another study by Rajaei (2017), out of 103 isolates studied, 26 (25.2%) strains showed high resistance to meropenem and imipenem, of which 19 (73%) isolates were MBL producer^[22].

In all of these reports, the rate of resistance to the antibiotics used in this study (ceftazidime, cefotaxime, and gentamicin in Imani's study, imipenem in Sadeghi's study, and ceftazidime and imipenem in Taghvaei's study) was lower than that found in this research, which could be due to the fact that those studies were performed almost a decade ago. In the study conducted in Ahvaz, colistin was reported as the most effective antibiotic against *Pseudomonas*, consistent with this research result.

Sadri et al. (2010) reported high levels of antibiotic resistance in Tehran as follows: gentamicin (86%), ceftazidime (73%), amikacin (73%), and ciprofloxacin (55%)^[23]; in their study, resistance to gentamicin, ceftazidime, and ciprofloxacin was higher than that found in this study. Their study results are not similar to this research findings, which could be due to factors such as statistical population, sample size, and type of diagnostic method used. Radan et al. (2016) showed multi-drug resistance in 38% of the isolates and extensive drug resistance in 62% of the isolates from burns in Isfahan. The highest resistance rate was observed to amikacin, cefepime, ceftazidime, ciprofloxacin, imipenem, meropenem, and tobramycin (more than 90% of the isolates), while the resistance rate to aztreonam and piperacillin/tazobactam was reported as 86 and 70.7%, respectively. The most effective antibiotics were colistin and polymyxin B, and *blaIMP* gene was detected in 74.3% of the metallo- β -lactamase producing isolates^[24]. Therefore, there is a high level of resistance to a variety of

antibiotics in Isfahan, and the prevalence of *blaIMP* gene is very high. However, colistin was found to be an effective antibiotic against *Pseudomonas*, consistent with the present study result.

Nikookar et al. (2013) showed the resistant rate of *P. aeruginosa* strains to imipenem to be 23.3% in Gilan province [25], which is less than the present study finding, suggesting further expansion of enzyme-producing strains in Gilan province over this period.

In this survey, out of 94 *P. aeruginosa* isolates, 44 strains were identified as β -lactamase and carbapenemase producers, among which 4 (4 of 44, 9.1%) samples were positive for *SIM* gene, and 7 (7 of 44, 15.9%) samples were positive for *AmpC* gene (4.3% and 7.4% in all the studied isolates, respectively).

Rafiee et al. (2014) reported the frequency of *AmpC* gene in all the studied isolates of *P. aeruginosa* to be 5% [26]. The frequency of *SIM* gene in 6771 samples was shown to be 14% by Alhaki et al. (2017) [27]. In another study, the frequency of *blaSIM* gene in 120 samples was determined as 17.1% by Haghi et al. (2017) [28]. Adabi et al. (2017) showed that out of 100 samples, 20 (20%) samples were positive for *AmpC* gene [29]. The frequency of *AmpC* gene in 114 samples was shown to be 85% by Tahmasebi et al. (2018) [30]. In the present study, the frequency of *SIM* and *AmpC* genes was reported as 4.3 and 7.4%, respectively. Except for Rafiee's study, the frequency of *SIM* and *AmpC* genes in this study was less than that reported in the previous studies. The discrepancy in this and other studies results could be due to geographical variation, so that even in some cases, variations in reports from various hospitals in the same city may be quite persistent and extensive [31]. It is recommended that further studies be

performed to investigate the presence of other MBL coding genes and to determine the genetic origin of these genes in clinical isolates of *P. aeruginosa*.

Conclusion

This study results indicated a high prevalence of drug resistance in clinical isolates of *P. aeruginosa* collected from Gilan hospitals. In particular, there was an increasing rate of resistance to beta-lactam antibiotics, and the presence of MBL and ESBL associated genes was considerable. This limits the choice of suitable treatment for patients with severe infections.

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Authors Contribution: Conceptualization: LA, SFK; Data curation and formal analysis: LA, EH; Funding acquisition: SFK; Investigation: SFK; Methodology and project administration: LA; Resources: LA, SFK; Software: LA, EH; Supervision: LA; Validation: LA, EH; Visualization: EH; Writing of original draft: EH, SFK; Writing, reviewing, and editing: EH.

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References

1. Arabestani MR, Rajabpour M, Yousefi Mashouf R, Alikhani MY, Mousavi SM.

- Expression of efflux pump MexAB-OprM and OprD of *Pseudomonas aeruginosa* strains isolated from clinical samples using qRT-PCR. *Arch Iran Med.* 2015; 18(2):102-8.
2. Rajabpour M, Alikhani MY. MIC determination of *Pseudomonas aeruginosa* strains were isolated from clinical specimens of patients admitted to educational hospitals in Hamedan (90-91). *Iran J Microbiol.* 2013; 7(3):18-25.
 3. Wong YP, Chua KH, Thong KL. One-step species-specific high-resolution melting analysis for nosocomial bacteria detection. *J Microbiol Methods.* 2014; 107:133-7.
 4. Brooks GF, Carroll KC, Butel JS. Jawetz, Melnick & Adelberge's Medical microbiology. 25th ed. London: Mc GrawHill; 2010, 253-6
 5. Akpaka PE, Legal B, Padman J. Molecular detection and epidemiology of and extended-spectrum beta-lactamase genes prevalent in clinical isolates of *Klebsiella pneumoniae* and *E.coli* from Trinidad and Tobago. *West Indian Med J.* 2010; 59 (6):591-6.
 6. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother.* 2010; 54 (3):969-76.
 7. Shahcheraghi F, Shakibaie MR, Noveiri H. Molecular identification of ESBL genes blaGES-1, blaVEB-1, blaCTX-M, blaOXA-1, blaOXA-4, blaOXA-10, and blaPER-1 in *Pseudomonas aeruginosa* strains isolated from burn patients by PCR, RFLP and sequencing techniques. *Int J Bioeng Life Sci.* 2010; 4 (1):138-42.
 8. Walsh TR, Bolmstrom A, Qwarnstrom A, Gales A. Evaluation of a new Etest for detection metallo-beta-lactamases in routine clinical testing. *J Clin Microbiol.* 2002; 40(8):2755-9.
 9. Poirel L, Guibert M, Girlich D, Naas T, Nordmann P. Cloning, sequence analyses, expression, and distribution of ampC-ampR from *Morganella morganii* clinical isolates. *Antimicrob Agents Chemother.* 1999; 43(4):769-76.
 10. Pitout JD, Chow BL, Gregson DB, Laupland KB, Elsayed S, Church DL. Molecular epidemiology of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* in the Calgary Health Region: Emergence of VIM2-producing isolates. *J Clin Microbiol.* 2007; 45(2):294-8.
 11. Hall GS. Bailey & Scott's Diagnostic Microbiology. The Oxford University; Press, 2013.
 12. Altaai ME, Aziz IH, Marhoon AA. Identification *Pseudomonas aeruginosa* by 16s rDNA gene for differentiation from other *Pseudomonas* species that isolated from patients and environment. *Baghdad Sci J.* 2014; 11(2):1028-34.
 13. Khosravi AD, Moloudsadat Motahar M, Abbasi Montazeri E. The frequency of Class1 and 2 integrons in *Pseudomonas aeruginosa* strains isolated from burn patients in a burn center of Ahvaz, Iran. *PLoS One.* 2017; 12(8):e0183061.
 14. Cayci YT, Coban AY, Gunaydin M. Investigation of plasmid-mediated quinolone resistance in *Pseudomonas aeruginosa* clinical isolates. *Indian J Med Microbiol.* 2014; 32(3):285-9.
 15. Fazeli N, Momtaz H. Virulence gene profiles of multidrug-resistant *Pseudomonas aeruginosa* isolated from Iranian hospital infections. *Iran Red Crescent Med J.* 2014; 16(10):e15722.
 16. 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 Korean university hospital and comparison of screening methods for detecting metallo-beta-lactamase. *J Microbiol Methods*. 2003; 54(3):411-8.
17. Gendrin C, Contreras-Martel C, Bouillot S, Elsen S, Lemaire D, Skoufias DA, et al. Structural basis of cytotoxicity mediated by the type III secretion toxin ExoU from *Pseudomonas aeruginosa*. *PLoS Pathog*. 2012; 8(4):e1002637.
 18. Imani Foolad A, Rostami Z, Shapouri R. Antimicrobial resistance and ESBL prevalence in *Pseudomonas aeruginosa* strains isolated from clinical specimen by phenotypic and genotypic methods. *J Ardabil Univ Med Sci*. 2010; 10(3):189-98.
 19. 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.
 20. Taghvaei R, Shojapour M, Sadeghi A, Pourbabaie AA. The study of antibiotic resistance pattern and the frequency of extended-spectrum beta-lactamases (ESBL) in *Pseudomonas aeruginosa* strains isolated from medical centers in Arak city, Iran. *Qom Univ Med Sci J*. 2013; 7(4):36-41
 21. Mardaneh J, Ahmadi K, Jahan Sepas A. Determination antimicrobial resistance profile of *Pseudomonas aeruginosa* strains isolated from hospitalized patients in Taleghani hospital (Ahvaz, Iran) from 2011-2012. *J Fasa Univ Med Sci*. 2013; 3(3):188-93.
 22. Rajaei S, Kazemi-Pour N, Rokhbakhsh-Zamin F. Frequency of plasmid-mediated quinolone resistance genes among clinical isolates of *Pseudomonas aeruginosa* in Kerman, Iran. *Iran J Med Microbiol*. 2017; 11(3):10-8.
 23. 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.
 24. Radan M, Moniri R, Khorshidi A, Gilasi H, Norouzi Z, Beigi F, et al. Emerging carbapenem-resistant *Pseudomonas aeruginosa* isolates carrying blaIMP among burn patients in Isfahan, Iran. *Arch Trauma Res*. 2016; 5(3):e33664.
 25. Nikookar I, Tishayar A, Flakiyan Z, Alijani K, Rehana-banisaheed S, Hossinpour M, et al. Antibiotic resistance and frequency of Class 1 integrons among *Pseudomonas aeruginosa* isolated from burn patients in Gilan, Iran. *Iran J Microbiol*. 2013; 5(1):36-41
 26. Rafiee R, Eftekhari F, Tabatabaei SA, Minaee Tehrani D. Prevalence of extended-spectrum and metallo- β -lactamase production in AmpC β -lactamase producing *Pseudomonas aeruginosa* isolates from burns. *Jundishapur J Microbiol*. 2014; 7(9):e16436.
 27. Alhaki T, Sadeghi Fard N, Saye Miri K, Bimanand L, Azizian R, Tabasi M, et al. The resistance rate of metallo-beta-lactamases among *Pseudomonas aeruginosa* isolates in Iran: A meta-analysis study. *J Infect Trop Dis*. 2017; 76:1-14.
 28. Hagi F, Keramati N, Hemmati F, Zeighami H. Distribution of integrons and gene cassettes among metallo- β -lactamase producing *Pseudomonas aeruginosa* clinical isolates. *Infect Epidemiol Microbiol*. 2017; 3(2):36-40.
 29. Adabi J, Shahraki Zahedani S, Bokaeian M, Tahmasebi H. An investigation of the prevalence of AmpC-producing *Pseudomonas aeruginosa* in clinical

- samples in Zahedan city, Iran. Qom Univ Med Sci J. 2017; 11(4):61-71.
30. Tahmasebi H, Alikhani MY, Dehbashi S, Arabestani MR. Investigation of the relationship between the presence of chromosomal and plasmid-encoded AmpC genes and type of clinical specimen in *Pseudomonas aeruginosa*. J Babol Univ Med Sci. 2018; 20(3):36-43.
31. Wassef M, Behiry I, Younan M, El Guindy N, Mostafa S, Abada E. Genotypic identification of AmpC β -lactamases production in gram-negative bacilli isolates. Jundishapur J Microbiol. 2014; 7(1):e8556.
32. Aksoy MD, Çavuşlu Ş, Tuğrul HM. Investigation of metallo beta lactamases and oxacilinases in carbapenem resistant *Acinetobacter baumannii* strains isolated from inpatients. Balkan Med J. 2015; 32(1):79-83.