Jundishapur J Microbiol. 2021 July; 14(7):e115977. doi: 10.5812/jjm.115977.

Published online 2021 September 13. **Research Article**

Molecular Detection of Carbapenemases and Extended-Spectrum β-Lactamases-Encoding Genes in Clinical Isolates of *Pseudomonas aeruginosa* in Iran

Esmaeil Rahimi [1], Ali Asgari [2], Taher Azimi [3] and Saeed Soleiman-Meigooni [1,*]

[1] Infectious Diseases Research Center, AJA University of Medical Sciences, Tehran, Iran
[2] Department of Infectious Diseases, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran
[3] Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[*] *Corresponding author*: Infectious Diseases Research Center, AJA University of Medical Sciences, 1411718541, Tehran, Iran. Email: dr.saeed.meigooni@gmail.com

Received 2021 May 15; **Revised** 2021 August 14; **Accepted** 2021 August 15.

Abstract

Background: *Pseudomonas aeruginosa* is a unique Gram-negative opportunistic pathogen that is the leading cause of nosocomial infections.
Objectives: This study aimed to investigate the prevalence of the main carbapenemase and extended-spectrum β-lactamases encoding genes in *P. aeruginosa* clinical isolates.
Methods: In the present study, we collected 85 *P. aeruginosa* clinical isolates from different wards of three military hospitals in Tehran, Iran. We used disk diffusion and agar dilution methods to determine resistance to 12 different antibiotics in these isolates. Also, we assessed the bla_{IMP}, bla_{VIM}, bla_{SHV}, bla_{TEM}, and bla_{CTX} genes by polymerase chain reaction methods among all isolates.
Results: Our results revealed that all isolates were resistant to two antibiotics, and 76 (89.4%) of isolates were multidrug-resistant. We observed maximum and minimum resistance rates against ticarcillin (n = 77; 90.5%) and colistin (n = 7; 8.2%), respectively. The bla_{VIM}, bla_{IPM}, bla_{TEM}, bla_{SHV}, and bla_{CTX} genes were harbored by 44 (51.8%), 20 (23.5%), 41 (48.2%), 24 (28.2%), and 16 (18.8%) isolates, respectively.
Conclusions: The resistance rate among *P. aeruginosa* strains is significantly increasing that causes nosocomial infections due to different mechanisms, including the high frequency of metallo-β-lactamases and extended-spectrum β-lactamases genes.

Keywords: bla_{VIM}, Extended-Spectrum β-lactamases, *Pseudomonas aeruginosa*

1. Background

Pseudomonas aeruginosa is a unique Gram-negative opportunistic pathogen that causes a broad range of complicated-to-treat infections that resist antibiotic therapies (1, 2). *Pseudomonas aeruginosa* has the remarkable capability to develop resistance to a broad class of antibiotics and is associated with significant morbidity and mortality (3, 4). Multidrug-resistance (MDR) in *P. aeruginosa* is associated with the prevalence of different genes, especially those encoding class B carbapenemases [Metallo-β-lactamases (MBLs)] and extended-spectrum β-lactamases (ESBLs) (5, 6). Since MBL and ESBL-producing bacteria are often MDR, the treatment of infections caused by these bacteria is very challenging (7). Clinicians have to use polymyxins, such as colistin and polymyxin B, as a last-line antimicrobial agent. However, recently published studies have reported that resistance to polymyxins, especially colistin, is increasing due to the spread of *mcr-1* and other *mcr* genes (8).

Pseudomonas aeruginosa can acquire resistance to carbapenems and β-lactams with the production of carbapenemases, including the Verona integron-encoded β-lactamase (VIM), imipenemase (IMP), and ESBLs such as the TEM, SHV, and CTX-M type enzymes (9-11). Carbapenemase and ESBL enzymes can hydrolyze broad-spectrum β lactam antibiotics, including imipenem, meropenem, cephalosporins, cefotaxime, ceftazidime, and monobactams (12, 13). Ambler class B enzymes, such as VIM and IMP, are the most frequent enzymes related to the carbapenemases-mediated resistance mechanism, and in the case of ESBLs, TEM, SHV, and CTX-M types are the vast majority of enzymes among Gram-negative bacilli (7, 14).

2. Objectives

Given the importance of increasing resistance mediated by carbapenemases and ESBLs, this study aimed to

investigate the prevalence of the main carbapenemases (bla_{VIM} and bla_{IPM}) and ESBLs (bla_{TEM}, bla_{SHV}, and bla_{CTX}) encoding genes in *P. aeruginosa* clinical isolates collected from three military hospitals in Tehran, Iran.

3. Methods

3.1. Bacterial Isolates

In this cross-sectional study, we collected 85 *P. aeruginosa* isolates from hospitalized military patients in different wards of three university hospitals [501 Hospital (Imam Reza), Khanevadeh Artesh Hospital, and Besat General Hospital] in Tehran, Iran, from 2019 to 2020 (Table 1). At the first step, all bacterial samples were cultured on common bacterial growth media, including blood agar, MacConkey agar, and Tryptic Soy Broth, and incubated at 37°C for 18 to 24 hours. After incubation, the conventional biochemical and microbiologic tests, including Gram stain, catalase, and oxidase test, growth at 42°C, growth on triple sugar iron agar and Kligler iron agar, pigment production on Mueller-Hinton agar (Merck Co., Germany), IMVIC (Indole, Methyl red, Voges proskauer, and Citrate) test, and motility test were used to identify the isolates (2, 15). We stored the isolates at -70°C in Trypticase soy broth (TSB) containing 20% glycerol until molecular analysis.

3.2. Antimicrobial Susceptibility Test

We carried out antimicrobial susceptibility testing using the disk diffusion method on Mueller-Hinton agar (Merck) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Here, the susceptibility of the isolates to the 12 antimicrobial agents of imipenem (IMI; 10 μg), meropenem (MEM; 10 μg), cefepime (CPM; 30 μg), cefotaxime (CTX; 30 μg), ceftazidime (CAZ; 30 μg), piperacillin (PRL; 100 μg), piperacillin/tazobactam (PTZ; 100/10 μg), ticarcillin (TIC; 75 μg), ciprofloxacin (CIP; 5 μg), gentamicin (GM; 10 μg), amikacin (AK; 30 μg), and colistin sulfate (CO; 10 μg) (Mast, UK) was determined. We classified the isolates resistant to at least three different antimicrobial agents as MDR (15). We also used the agar dilution method to determine the minimum inhibitory concentrations (MICs) of gentamicin, imipenem, ciprofloxacin, piperacillin, and colistin (16). The interpretation of MIC results was carried out according to the CLSI breakpoints (imipenem and colistin: susceptible: $\leq$ 2 μg/mL, resistant: $\geq$ 8 μg/mL, gentamicin: susceptible: $\leq$ 4 μg/mL, resistant: $\geq$ 16 μg/mL, piperacillin: susceptible: $\leq$ 16 μg/mL, resistant: $\geq$ 128 μg/mL, ciprofloxacin: susceptible: $\leq$ 1 μg/mL, resistant: $\geq$ 4 μg/mL) (17). We defined MIC as the lowest concentration of an antimicrobial agent that inhibited the bacteria's growth compared with *P. aeruginosa* PAO1 as the positive control.

3.3. Molecular Detection of Genes

According to the manufacturer, we extracted all the collected isolates' whole genomic DNA using DNJia Plus Tissue and Bacteria Kit's (ROJE Technologies Co, Iran: Cat. No.: DN983051) protocol. Specific primers (Table 1) for the bla_{IPM}, bla_{VIM}, bla_{SHV}, bla_{TEM}, bla_{CTX}, and *mcr-1* genes were used for molecular detection using polymerase chain reaction (PCR) methods. The primers and their thermal cycling protocols used in this study were described previously (18-21). We carried out DNA amplification on a thermal cycler (Eppendorf, Mastercycler Gradient; Eppendorf, Hamburg, Germany) under the following condition: (1) 10 - 20 ng of extracted sample DNA; (2) 1 μL of each primer (10 pmol); (3) 12.5 μL of Taq DNA Polymerase 2x Master Mix RED (Ampliqon Co, Denmark; Cat. No.: A180301); and (4) up to 25 μL distilled water. Polymerase chain reaction conditions were set based on a previously published study by Azimi et al. (20). We assessed all PCR products by electrophoresis on 1% agarose gel (SinaClon, Iran). Escherichia coli ATCC 25922 was used as a positive control for the PCR assay.

4. Results

4.1. Number and Distribution of Isolates

From 2019 to 2020, 85 *P. aeruginosa* clinical strains were collected from three university hospitals [501 Hospital (Imam Reza), Khanevadeh Artesh Hospital, and Besat General Hospital] in Tehran, Iran. The distribution of *P. aeruginosa* isolates in different hospital wards and among different clinical samples is shown in Table 2. The patients' age ranged from 1 to 89 years (56.5 $\pm$ 22), and approximately 56.5 and 43.5% of the *P. aeruginosa* isolates belonged to female and male samples, respectively. Our analyses revealed that most *P. aeruginosa* isolates were identified in the ICU (n = 34; 40%) and surgery ward (n = 16; 18.8%), respectively. Moreover, the results revealed that most *P. aeruginosa* isolates were isolated from wound and urine samples, respectively.

4.2. Antimicrobial Susceptibility Test

Table 3 summarizes the results of the disk diffusion test and the agar dilution method. Our results showed that all the isolates were resistant to two antibiotics, and 76 (89.4%) of isolates were MDR. The maximum and minimum resistance rates were against ticarcillin (n = 77; 90.5%) and colistin (n = 7; 8.2%), respectively. In carbapenem antibiotics, 76 (89.4%) isolates were resistant to at least imipenem or meropenem or both of them. Resistance to the cephalosporins group was higher (n = 79; 92.9%) than carbapenems. Data from MIC assay showed that among the five tested antibiotics, isolates had higher resistance to ciprofloxacin (n = 63; 74.1%). In general, our findings showed that from 34 *P. aeruginosa* isolated from the ICU, 21

Table 1. Polymerase Chain Reaction Primers Used for Amplification of Antimicrobial Resistance Genes

Genes	Primer Sequences	Product Size (bp)
bla_{IMP}	F: 5'- GGAATAGAGTGGCTTAATTCTC-3'; R: 5'- CCAAACCACTACGTTATCT-3'	189
bla_{VIM}	F: 5'-ATGGTGTTTGGTCGCATATC-3'; R: 5'-TGGGCCATTCAGCCAGATC-3'	510
bla_{TEM}	F: 5'-ATGAGTATTCAACATTTCCG-3'; R: 5'-CTGACAGTTACCAATGCTTA-3'	867
bla_{SHV}	F: 5'-GATGAACGCTTTCCCATGATG -3'; R: 5'-CGCTGTTATCGCTCATGGTAA -3'	214
bla_{CTX}	F: 5'-TTTGCGATGTGCAGTACCAGTAA -3'; R: 5'-CGATATCGTTGGTGGTGCCATA -3'	590
mcr-1	F: 5'- CGGTCAGTCCGTTTGTTC-3'; R: 5'- CTTGGTCGGTCTGTAGGG-3'	309

Table 2. Epidemiological and Clinical Characteristics of the *Pseudomonas aeruginosa* Isolates

Variables	No.	%
Sex		
Females	48	56.5
Males	37	43.5
Ward		
Intensive care unit (ICU)	34	40
Surgery	16	18.8
Orthopedic	5	5.9
Internal	11	12.9
Emergency	14	16.5
General	5	5.9
Origin		
Wound	28	32.9
Urine	23	27
Tracheal aspirate	14	16.5
Blood	9	10.6
Sputum	6	7
Fluid	3	3.6
Stool	2	2.4
Total	85	100

(61.8%) *P. aeruginosa* isolates were resistant to meropenem and cefepime. Moreover, 12 (75%) *P. aeruginosa* isolates collected from the surgery ward were resistant to cefotaxime and ticarcillin. On the other hand, 21 (75%), 16 (69.5%), and 10 (71.4%) of *P. aeruginosa* isolates recovered from wound, urine, and tracheal aspirate samples were resistant to ticarcillin, respectively. Results showed that in comparison to other clinical samples, resistance rates to meropenem (82.1%), cefotaxime (67.8%), and amikacin (64.2%) among *P. aeruginosa* isolated from wound samples were high.

4.3. Distribution of MBL and ESBL-Producing Isolates

We carried out the PCR technique to detect bla_{IPM}, bla_{VIM}, bla_{SHV}, bla_{TEM}, and bla_{CTX}, genes in all *P. aeruginosa* isolates. The frequency of this genes was as follow:

- MBL genes: bla_{VIM} (n = 44; 51.8%), bla_{IPM} (n = 20; 23.5%).

- ESBL genes: bla_{TEM} (n = 41; 48.2%), bla_{SHV} (n = 24; 28.2%), and bla_{CTX} (n = 16; 18.8%). The frequency rates of MBL- and ESBL-producing isolates in this study were 56 (65.9%) and 60 (70.6%), respectively. Among all the isolates, 81 (95.3%) had at least one of the studied genes. Table 4 shows the distribution of these genes among all the *P. aeruginosa* strains collected in this study. Data analysis showed that the presence of the bla_{IMP} and bla_{VIM} genes was significantly associated with resistance to carbapenems ($P < 0.002$). Also, for ESBL genes, namely bla_{TEM}, bla_{SHV}, and bla_{CTX}, this association was observed for cephalosporins ($P < 0.007$), including cefepime, cefotaxime, and ceftazidime. Due to the resistance to colistin observed in seven isolates, we also carried out PCR to detect the mcr-1 gene, but we did not find it in any isolates. Our analyses revealed that in comparison to other hospital wards, the frequency of bla_{VIM} and bla_{TEM} genes among the *P. aeruginosa* isolates collected from ICU and emergency wards was high. On the other hand, the frequency of these genes among *P. aeruginosa* strains isolated from the surgery, and internal wards was low. Moreover, the frequency of bla_{VIM} and bla_{TEM} genes among *P. aeruginosa* strains isolated from wound and urine samples was high.

5. Discussion

Metallo-β-lactamases- and ESBL-producing isolates of *P. aeruginosa* are the most frequent pathogen causing infections in hospitalized patients. Acquired resistance due to the widespread use of antibiotics, especially in clinical treatments, increases antimicrobial resistance in pathogenic bacteria with emerging MDR and extensively drug-resistant (XDR) and pan drug-resistant (PDR) strains (22, 23). Multidrug-resistant mechanisms of *P. aeruginosa* are mainly due to ß-lactamase production, efflux pumps, and outer membrane proteins changes (24). This study aimed to evaluate the prevalence of MBL, and ESBL genes in *P. aeruginosa* isolates recovered from patients in different wards of three military hospitals in Tehran, Iran. Data analysis showed that most of the isolates were MDR (89.4%), and the highest resistance was observed for ticarcillin (90.5%), followed by meropenem (78.8%) and ciprofloxacin (76.5%). As previous studies reported, the rate of resistance to antimicrobial agents varies in different areas of Iran, and antibiotics resistance has arisen significantly in *P. aeruginosa* over the past two decades (25).

Various studies have surveyed the prevalence of antibiotic resistance among *P. aeruginosa* strains. The re-

Table 3. Antimicrobial Resistance Profile of the *Pseudomonas aeruginosa* Isolates [a]

Antibiotics	Disk Diffusion			Agar Dilution		
	S	I	R	S	I	R
Imipenem	39 (48.2)	-	44 (51.8)	19 (29.5)	7 (8.2)	54 (62.3)
Meropenem	18 (21.2)	-	67 (78.8)	-	-	-
Cefepime	23 (27.1)	1 (1.2)	61 (71.7)	-	-	-
Cefotaxime	26 (30.6)	-	59 (69.4)	-	-	-
Ceftazidime	32 (37.7)	-	53 (62.3)	-	-	-
Ticarcilli	8 (9.5)	-	77 (90.5)	-	-	-
Piperacillin	29 (34.1)	1 (1.2)	55(64.7)	22 (26.9)	2 (2.4)	61 (71.7)
Ciprofloxacin	20 (23.5)	-	65 (76.5)	19 (22.4)	3 (3.5)	63 (74.1)
Piperacillin/tazobactam	30 (35.3)	7 (8.2)	48 (56.5)	-	-	-
Gentamicin	28 (33)	-	57 (67)	25 (29.4)	1 (1.2)	59 (69.4)
Amikacin	28 (33)	3 (3.5)	54 (63.5)	-	-	-
Colistin sulfate	78 (91.8)	-	7 (8.2)	74 (89.4)	2 (2.4)	7 (8.2)

Abbreviations: S, sensitive; I, intermediate; R, resistant.
[a] Values are expressed as No. (%).

sults of a study performed by Azimi et al. revealed that *P. aeruginosa* had the lowest and highest resistance rates to levofloxacin and ticarcillin-clavulanic acid, respectively (15). Labaste et al. revealed that 20.5% of *P. aeruginosa* strains developed carbapenem resistance (26). Emaneini et al. reported that polymyxin B, piperacillin/tazobactam and meropenem were the most active antibiotics against *P. aeruginosa* isolates (27). In general, antimicrobial susceptibility and MIC test results are relatively similar to previous findings obtained by Farshadzadeh et al. from Iran, Goli et al. from Iran, and Adjei et al. from South Africa (28-30). However, we found more variation in resistance rates than in other studies (23, 31, 32). These differences in resistance rates are probably because of the accumulation of resistance to that area's antimicrobial agents.

Among all isolates, 95.3% had at least one of the studied genes. The most frequent genes in this study were bla_{VIM} (n = 44; 51.8%) and bla_{TEM} (n = 41; 48.2%), respectively. Also, our data showed that 65.9% and 70.6% of isolates were positive for MBL- and ESBL-producing genes. In a study by Salimi and Eftekhar on 32 carbapenem-resistant, MBL-producing *P. aeruginosa* isolates, 56.25 and 46.8% of the isolates were positive for bla_{IMP} and bla_{VIM}, respectively (33). However, in our results, the carriage for the bla_{VIM} gene was more than two times higher than for bla_{IMP}. Aghamiri et al. reported that the frequency of bla_{VIM} (33%) was higher than $bla_{IP}M$ (9%), but their frequencies were lower than our results (34). In another study, Shahcheraghi et al. reported that 68% of MBL-positive strains of *P. aeruginosa* harbored bla_{VIM}, but none was bla_{IMP}-positive (35). In conflict with our findings, Radan et al. reported that 74.3% of MBL isolates were positive for the bla_{IMP} gene (36).

In the case of ESBL genes, bla_{TEM}, bla_{SHV}, and bla_{CTX} genes vary in different studies. Our results, like other studies in Iran, showed that the most prevalent ESBL gene was bla_{TEM}. Pakbaten Toupkanlou et al. reported that among ESBL-positive strains of *P. aeruginosa*, the frequency of the bla_{TEM} and bla_{SHV} genes was 50% (22). Another report by Bokaeian et al. revealed that the bla_{TEM} and bla_{SHV} genes were harbored by 100 and 6.6% of ESBL-positive strains of *P. aeruginosa*, respectively (37). Bahrami et al. also reported that the prevalence rates of bla_{TEM}, bla_{SHV}, and bla_{CTX-M} were 57.29, 23.08, and 23.95% among 96 isolates of *P. aeruginosa* (6).

5.1. Conclusion

Our results indicated that resistance to almost all available antibiotics used in clinical settings against *P. aeruginosa* is significantly increasing. *Pseudomonas aeruginosa* resistance to antimicrobial agents is due to several mechanisms, but MBL- and ESBL genes cause resistance to the most frequently used antibiotics against this pathogen. In sum, our results indicated that the frequency rates of MBL- and ESBL genes are high among *P. aeruginosa* isolates, which could be the reason for the increased resistance to antimicrobial agents.

Acknowledgments

We would like to thank the Iranian Institute of Cell and Gene Therapy (Tehran, Iran) for their support.

Table 4. Frequencies and Distribution of MBL and ESBL Genes in 85 *Pseudomonas aeruginosa* Isolates

Resistance Genes	No. (%)
bla_{VIM}	44 (51.8)
bla_{IPM}	20 (23.5)
bla_{TEM}	41 (48.2)
bla_{SHV}	24 (28.2)
bla_{CTX}	16 (18.8)
Combination of genes [a]	
bla_{VIM}	11 (12.9)
bla_{IPM}	7 (8.2)
bla_{TEM}	6 (7)
bla_{SHV}	6 (7)
bla_{CTX}	3 (3.5)
$bla_{VIM} + bla_{IPM}$	3 (3.5)
$bla_{VIM} + bla_{TEM}$	13 (15.3)
$bla_{VIM} + bla_{SHV}$	4 (4.7)
$bla_{VIM} + bla_{CTX}$	3 (3.5)
$bla_{IPM} + bla_{TEM}$	2 (2.4)
$bla_{IPM} + bla_{CTX}$	1 (1.2)
$bla_{TEM} + bla_{SHV}$	5 (5.9)
$bla_{TEM} + bla_{CTX}$	2 (2.4)
$bla_{SHV} + bla_{CTX}$	1 (1.2)
$bla_{VIM} + bla_{IPM} + bla_{TEM}$	2 (2.4)
$bla_{IPM} + bla_{TEM} + bla_{CTX}$	3 (3.5)
$bla_{VIM} + bla_{TEM} + bla_{SHV}$	5 (5.9)
$bla_{VIM} + bla_{IPM} + bla_{SHV}$	1 (1.2)
$bla_{TEM} + bla_{SHV} + bla_{CTX}$	2 (2.4)
$bla_{VIM} + bla_{IPM} + bla_{TEM} + bla_{CTX}$	1 (1.2)
None of studied genes	4 (4.7)

[a] Other combination of genes was not detected in this study.

Footnotes

Authors' Contribution: Study concept and design, E.R. and S.S.M.; Analysis and interpretation of data, E.R.; Drafting of the manuscript, E.R.; Critical revision of the manuscript for important intellectual content, S.S.M., A.A., and T.A.; Statistical analysis, E.R.

Conflict of Interests: The authors declare that they have no conflicts of interest.

Data Reproducibility: The data presented in this study are openly available in one of the repositories or will be available on request from the corresponding author by this journal representative at any time during submission or after publication. Otherwise, all consequences of possible withdrawal or future retraction will be with the corresponding author.

Ethical Approval: The present study was approved by the Institutional Ethics Committee of the AJA University of Medical Sciences, with reference number IR.AJAUMS.REC.1399.181.

Funding/Support: This study was funded by the Aja University of Medical Sciences.

References

1. Skurnik D, Roux D, Cattoir V, Danilchanka O, Lu X, Yoder-Himes DR, et al. Enhanced in vivo fitness of carbapenem-resistant oprD mutants of Pseudomonas aeruginosa revealed through high-throughput sequencing. *Proc Natl Acad Sci USA*. 2013;**110**(51):20747–52. doi: 10.1073/pnas.1221552110. [PubMed: 24248354]. [PubMed Central: PMC3870709].
2. Azimi L, Fallah F, Karimi A, Shirdoust M, Azimi T, Sedighi I, et al. Survey of various carbapenem-resistant mechanisms of Acinetobacter baumannii and Pseudomonas aeruginosa isolated from clinical samples in Iran. *Iran J Basic Med Sci*. 2020;**23**(11):1396–400. doi: 10.22038/IJBMS.2020.44853.10463. [PubMed: 33235696]. [PubMed Central: PMC7671419].
3. Mihani F, Khosravi A. [Isolation of Pseudomonas aeruginosa strains producing metallo beta lactamases from infections in burned patients and identification of blaIMP and blaVIM genes by PCR]. *Iran J Med Microbiol*. 2007;**1**(1):23–31.
4. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: Mechanisms and alternative therapeutic strategies. *Biotechnol Adv*. 2019;**37**(1):177–92. doi: 10.1016/j.biotechadv.2018.11.013. [PubMed: 30500353].
5. 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-beta-lactamase. *J Microbiol Methods*. 2003;**54**(3):411–8. doi: 10.1016/s0167-7012(03)00090-3. [PubMed: 12842488].
6. Bahrami M, Mmohammadi-Sichani M, Karbasizadeh V. Prevalence of SHV, TEM, CTX-M and OXA-48 β-lactamase genes in clinical isolates of Pseudomonas aeruginosa in Bandar-Abbas, Iran. *Avicenna J Clin Microbiol Infect*. 2018;**5**(4):86–90. doi: 10.34172/ajcmi.2018.18.
7. Naas T, Cuzon G, Bogaerts P, Glupczynski Y, Nordmann P. Evaluation of a DNA microarray (Check-MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases. *J Clin Microbiol*. 2011;**49**(4):1608–13. doi: 10.1128/JCM.02607-10. [PubMed: 21325547]. [PubMed Central: PMC3122838].
8. Pungcharoenkijkul S, Traipattanakul J, Thunyaharn S, Santimaleeworagun W. Antimicrobials as single and combination therapy for colistin-resistant Pseudomonas aeruginosa at a university hospital in Thailand. *Antibiotics*. 2020;**9**(8). doi: 10.3390/antibiotics9080475. [PubMed: 32756327]. [PubMed Central: PMC7459651].
9. Liakopoulos A, Mavroidi A, Katsifas EA, Theodosiou A, Karagouni AD, Miriagou V, et al. Carbapenemase-producing Pseudomonas aeruginosa from central Greece: molecular epidemiology and genetic analysis of class I integrons. *BMC Infect Dis*. 2013;**13**:505. doi: 10.1186/1471-2334-13-505. [PubMed: 24168643]. [PubMed Central: PMC3870975].
10. Queenan AM, Bush K. Carbapenemases: The versatile beta-lactamases. *Clin Microbiol Rev*. 2007;**20**(3):440–58. doi: 10.1128/CMR.00001-07. [PubMed: 17630334]. [PubMed Central: PMC1932750].
11. Kiros T, Workineh L. The emergence of extended-spectrum β-lactamases and carbapenemases from none human sources: The challenges and opportunities. *J Bacteriol Mycol Open Access*. 2019;**7**(6):164–73.
12. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, et al. beta-lactamases and beta-lactamase inhibitors in the 21st century. *J Mol Biol*. 2019;**431**(18):3472–500. doi: 10.1016/j.jmb.2019.04.002. [PubMed: 30959050]. [PubMed Central: PMC6723624].
13. Sawa T, Kooguchi K, Moriyama K. Molecular diversity of extended-spectrum beta-lactamases and carbapenemases, and antimicrobial resistance. *J Intensive Care*. 2020;**8**:13. doi: 10.1186/s40560-020-0429-6. [PubMed: 32015881]. [PubMed Central: PMC6988205].

14. Fallah F, Azimi T, Azimi L, Karimi A, Rahbar M, Shirdoust M, et al. Evaluating the antimicrobial resistance and frequency of AmpC β-lactamases bla gene in Gram-negative bacteria isolates collected from selected hospitals of Iran: A multicenter retrospective study. *Gene Reports*. 2020;**21**:100868. doi: 10.1016/j.genrep.2020.100868.
15. Azimi T, Maham S, Fallah F, Azimi L, Gholinejad Z. Evaluating the antimicrobial resistance patterns among major bacterial pathogens isolated from clinical specimens taken from patients in Mofid Children's Hospital, Tehran, Iran: 2013-2018. *Infect Drug Resist*. 2019;**12**:2089–102. doi: 10.2147/IDR.S215329. [PubMed: 31410032]. [PubMed Central: PMC6645606].
16. Sahu C, Jain V, Mishra P, Prasad KN. Clinical and laboratory standards institute versus European committee for antimicrobial susceptibility testing guidelines for interpretation of carbapenem antimicrobial susceptibility results for Escherichia coli in urinary tract infection (UTI). *J Lab Physicians*. 2018;**10**(3):289–93. doi: 10.4103/JLP.JLP_176_17. [PubMed: 30078964]. [PubMed Central: PMC6052810].
17. CLSI. *Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement*. **31**. Wayne, USA: Clinical and Laboratory Standards Institute; 2011.
18. Maspi H, Mahmoodzadeh Hosseini H, Amin M, Imani Fooladi AA. High prevalence of extensively drug-resistant and metallo beta-lactamase-producing clinical Acinetobacter baumannii in Iran. *Microb Pathog*. 2016;**98**:155–9. doi: 10.1016/j.micpath.2016.07.011. [PubMed: 27448835].
19. Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, et al. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a Pseudomonas aeruginosa clinical isolate in France. *Antimicrob Agents Chemother*. 2000;**44**(4):891–7. doi: 10.1128/AAC.44.4.891-897.2000. [PubMed: 10722487]. [PubMed Central: PMC89788].
20. Azimi T, Azimi L, Fallah F, Pourmand MR, Ostadtaghizadeh A, Abai MR, et al. Detection and characterization of Enterobacteriaceae family members carried by commensal Rattus norvegicus from Tehran, Iran. *Arch Microbiol*. 2021;**203**(4):1321–34. doi: 10.1007/s00203-020-02126-0. [PubMed: 33386421].
21. Tarafdar F, Jafari B, Azimi T. Evaluating the antimicrobial resistance patterns and molecular frequency of bla oxa-48 and bla GES-2 genes in Pseudomonas aeruginosa and Acinetobacter baumannii strains isolated from burn wound infection in Tehran, Iran. *New Microbes New Infect*. 2020;**37**:100686. doi: 10.1016/j.nmni.2020.100686. [PubMed: 32774866]. [PubMed Central: PMC7394744].
22. Pakbaten Toupkanlou S, Najar Peerayeh S, Pirhajati Mahabadi R. Class A and D extended-spectrum beta-lactamases in imipenem resistant Pseudomonas aeruginosa isolated from burn patients in Iran. *Jundishapur J Microbiol*. 2015;**8**(8). e18352. doi: 10.5812/jjm.18352v2. [PubMed: 26468357]. [PubMed Central: PMC4600860].
23. De Rosa A, Mutters NT, Mastroianni CM, Kaiser SJ, Gunther F. Distribution of carbapenem resistance mechanisms in clinical isolates of XDR Pseudomonas aeruginosa. *Eur J Clin Microbiol Infect Dis*. 2019;**38**(8):1547–52. doi: 10.1007/s10096-019-03585-0. [PubMed: 31152264].
24. Zahedi Bialvaei A, Rahbar M, Hamidi-Farahani R, Asgari A, Esmailkhani A, Mardani Dashti Y, et al. Expression of RND efflux pumps mediated antibiotic resistance in Pseudomonas aeruginosa clinical strains. *Microb Pathog*. 2021;**153**:104789. doi: 10.1016/j.micpath.2021.104789. [PubMed: 33556480].
25. Vaez H, Salehi-Abargouei A, Khademi F. Systematic review and meta-analysis of imipenem-resistant Pseudomonas aeruginosa prevalence in Iran. *Germs*. 2017;**7**(2):86–97. doi: 10.18683/germs.2017.1113. [PubMed: 28626739]. [PubMed Central: PMC5466827].
26. Labaste F, Grossac J, Bounes FV, Conil JM, Ruiz S, Seguin T, et al. Risk factors for acquisition of carbapenem-resistance during treatment with carbapenem in the intensive care unit: A prospective study. *Eur J Clin Microbiol Infect Dis*. 2019;**38**(11):2077–85. doi: 10.1007/s10096-019-03644-6. [PubMed: 31482416]. [PubMed Central: PMC6800833].
27. Emaneini M, Kalantar-Neyestanaki D, Jabalameli L, Hashemi M, Beigverdi R, Jabalameli F. Molecular analysis and antimicrobial resistance pattern of distinct strains of Pseudomonas aeruginosa isolated from cystic fibrosis patients in Iran. *Iran J Microbiol*. 2019;**11**(2):98–107. [PubMed: 31341563]. [PubMed Central: PMC6635311].
28. Farshadzadeh Z, Khosravi AD, Alavi SM, Parhizgari N, Hoveizavi H. Spread of extended-spectrum beta-lactamase genes of blaOXA-10, blaPER-1 and blaCTX-M in Pseudomonas aeruginosa strains isolated from burn patients. *Burns*. 2014;**40**(8):1575–80. doi: 10.1016/j.burns.2014.02.008. [PubMed: 24767142].
29. Goli HR, Nahaei MR, Rezaee MA, Hasani A, Samadi Kafil H, Aghazadeh M, et al. Contribution of mexAB-oprM and mexXY (-oprA) efflux operons in antibiotic resistance of clinical Pseudomonas aeruginosa isolates in Tabriz, Iran. *Infect Genet Evol*. 2016;**45**:75–82. doi: 10.1016/j.meegid.2016.08.022. [PubMed: 27562333].
30. Adjei CB, Govinden U, Moodley K, Essack SY. Molecular characterisation of multidrug-resistant Pseudomonas aeruginosa from a private hospital in Durban, South Africa. *S Afr J Infect Dis*. 2017;**33**(2):38–41. doi: 10.1080/23120053.2017.1382090.
31. 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. [PubMed: 25644798].
32. Abbas HA, El-Ganiny AM, Kamel HA. Phenotypic and genotypic detection of antibiotic resistance of Pseudomonas aeruginosa isolated from urinary tract infections. *Afr Health Sci*. 2018;**18**(1):11–21. doi: 10.4314/ahs.v18i1.3. [PubMed: 29977252]. [PubMed Central: PMC6016981].
33. Salimi F, Eftekhar F. Prevalence of blaIMP, and blaVIM gene carriage in metallo-beta-lactamase-producing burn isolates of Pseudomonas aeruginosa in Tehran. *Turk J Med Sci*. 2014;**44**(3):511–4. doi: 10.3906/sag-1302-67. [PubMed: 25558658].
34. Aghamiri S, Amirmozafari N, Fallah Mehrabadi J, Fouladtan B, Samadi Kafil H. Antibiotic resistance pattern and evaluation of metallo-beta lactamase genes including bla- IMP and bla- VIM types in Pseudomonas aeruginosa isolated from patients in Tehran hospitals. *ISRN Microbiol*. 2014;**2014**:941507. doi: 10.1155/2014/941507. [PubMed: 24944839]. [PubMed Central: PMC4040202].
35. 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. [PubMed: 20954442].
36. 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. doi: 10.5812/atr.33664. [PubMed: 27800466]. [PubMed Central: PMC5078761].
37. Bokaeian M, Shahraki Zahedani S, Soltanian Bajgiran M, Ansari Moghaddam A. Frequency of PER, VEB, SHV, TEM and CTX-M genes in resistant strains of Pseudomonas aeruginosa producing extended spectrum beta-lactamases. *Jundishapur J Microbiol*. 2015;**8**(1). e13783. doi: 10.5812/jjm.13783. [PubMed: 25789123]. [PubMed Central: PMC4350043].