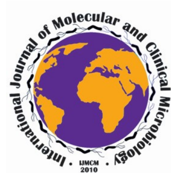
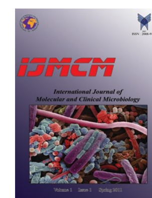
IJMCM 13(1) (2023) 1788-1794 Doi: 10.22034/ijmcm.2023.700074



International Journal of
Molecular and Clinical Microbiology



Resrach Article

# Plasmid-mediated Quinolone resistance genes in *Pseudomonas aeruginosa* isolates of burn infection

**Malahat Alinezhad [1], Maryam Mohammadi-Sichani [1*], Vajihe Karbasizadeh [2]**

*1 Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*
*2 Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

ARTICLE INFO

***Article history:***
Received 08 August 2022
Accepted 22 Feburary 2023
Available online 1 March 2023

***keywords:***
*Antibiotic resistance,*
*Burns,*
*Pseudomonas aeruginosa,*
*Resistance Factor,*
*Quinolones*

ABSTRACT

Plasmid-dependent resistance to quinolones is increasingly spreading among *P. aeruginosa* isolates worldwide. In this study, the evaluation of antibiotic resistance to quinolones in *P. aeruginosa* isolated from burn wound samples was investigated. In this descriptive study, 73 burn isolates of *P. aeruginosa* were collected. Antibiotic susceptibility of the isolates was assessed by the disk diffusion method. Then, the frequency of the *aac (6')-Ib, qnrS, qnrB,* and *qnrA* genes was determined by PCR. The highest resistance was related to the antibiotics Nalidixic acid (100%), Imipenem (98.7%), Ciprofloxacin (97.2%), Cefepime (100%), Meropenem (97.2%), Levofloxacin (100%), and Ofloxacin (100%). Among 73 *P. aeruginosa* isolates, 4 (5.5%) strains containing the *aac(6')-Ib* gene, 3 (4.1%) strains containing the *qnrA* gene, 3 (4.1%) strains with the *qnrB* gene, and 2 (7.2%) strains containing the *qnrS* gene were detected. In total, out of 73 strains of *P. aeruginosa* isolated from burns, 12 strains (16.5%) had one or four plasmids carrying quinolone resistance genes alone or simultaneously. The expansion of quinolone-resistant plasmid genes plays an important role in the prevalence of *P. aeruginosa* quinolone-resistant strains in burns, and control of Pseudomonas burn wound infections with quinolones has proven difficult.

## 1. Introduction

*Pseudomonas aeruginosa*, a Gram-negative bacillus, is an opportunistic pathogen that is considered one of the most important causes of nosocomial infections, especially in burns and immunocompromised individuals. Infections caused by *P. aeruginosa* in patients with immune deficiency are severe and life-threatening (Alhussain et al., 2021).

The burn infections commonly caused by *P. aeruginosa* which colonized by the patient's microflora or from the environment in burn wounds. Despite medical advances and intensive care for patients with burns, infection, is still the main cause of mortality in these patients; because burns not only damage the tissue, but also provide a suitable condition for the infectious agents. Therefore, the most important method for preventing complications and mortality in burned patients is rapid identification of the agent and antibiotic treatment (Theodorou et al., 2013; van Langeveld et al., 2017).

Unfortunately, *P. aeruginosa* has not only acquired an inherent resistance to a wide range of antibiotics but has also become a highly resistant bacterium by receiving various

*Corresponding authors: Maryam Mohammadi-Sichani
Email address: ma.mohammadi1347@iau.ac.ir

resistance genes. This bacterium has shown high resistance to a wide range of antibiotics, including beta-lactams, Tetracyclines, Chloramphenicols and erythromycins. Unnecessary prescription of antibiotics such as Piperacillin-Tazobactam, Imipenem, Meropenem, Amikacin, and Ciprofloxacin has led to the evolution of multiple resistance strains of *P. aeruginosa* (Barati et al., 2021). Fluoroquinolones are widely used in the treatment of burn infections. Fluoroquinolones inhibit DNA replication by inhibiting gyrase and preventing bacterial growth. Recently, resistance to fluoroquinolones has increased among bacterial pathogens due to the spread of *qnr* genes, including *qnr*A, *qnr*B, and *qnr*S (Juraschek et al., 2021).

The aim of this study was to isolate and identify *P. aeruginosa* strains in burn infection and to evaluate the frequency of quinolone resistance genes *aac (6')-Ib, qnrS, qnrB*, and *qnrA* in quinolone resistant strains by PCR.

## 2. Materials and Methods

In this experimental study, 73 isolates of *P. aeruginosa* were collected from clinical specimens obtained from the burn ward of Imam Musa Kazem Hospital in Isfahan, Iran. From the burn samples, 41 isolates (56.2%) were obtained from men and 32 isolates (43.8%) were obtained from women. All isolates of *P. aeruginosa* were identified by Gram staining and various biochemical tests, including the oxidase test, catalase test, OF test, growth at 42 °C, pyocyanin production in Müller-Hinton agar, and growth on TSI, EMB, and Cetrimide agar media (Koneman et al., 2006, Nahon et al., 2015).

### *2.1. Antibacterial Susceptibility testing*

Susceptibility to antibiotics was tested by the Kirby Bauer (disk diffusion) method using the CLSI recommendation (Bonev et al., 2008, Balouiri et al., 2016). The susceptibility of isolates to the antibiotics, including Nalidixic acid (NA, 30 µg), Ciprofloxacin (CIP, 5 µg), Levofloxacin (LE, 5 µg), Ofloxacin (OF, 5 µg), Imipenem (IPM, 10 µg), Meropenem (MRP, 10 µg) and Cefepime (CPM, 30 µg) was determined (Himedia, India).

### *2.2. Minimum inhibitory concentration (MIC) for Ciprofloxacin resistant quantification*

The MIC of Ciprofloxacin was determined by the microdilution broth method in 96-well microplates. According to the instructions of CLSI; first, the amount of antibiotic needed to make the stock solution was calculated using the following formula:

$$\text{Weight of powder (mg)} = \frac{\text{volume of solution (ml)} \times \text{concentration } (\mu g/ml)}{\text{potency of powder } (\mu g/mg)}$$

The lowest dilution of the drug at which no turbidity was observed was considered the MIC. The turbidity of the test wells was compared with the positive control well and their transparency of them was compared with the negative control well. Finally, the obtained results were compared with the standards available in CLSI. The *P. aeruginosa* ATCC: 27853 was used as control.

### *2.3. DNA Extraction and PCR Amplification*

The colony PCR technique was used in this study (Gholami et al., 2016). In order to amplify the desired fragment to investigate the presence of qnrS, qnrB, qnrA, and *aac(6')-Ib genes* specific primers were used according to Table 1 (Kao et al., 2016). The primers were synthesized by FAZA Biotech Co. (Tehran, Iran). The PCR reaction mixture consisted of the following values: one loop-full bacterial colony, 2.5 µl of 10X PCR buffer, 0.75 µl of 50 mM $MgCl_2$, 0.5 µl of 10 mM dNTP, 1 µl of each 10 pM primer, 0.25 µl of 5 U/µl Taq DNA polymerase enzyme (Sinaclon Co.), and finally 19 microliters of sterile distilled water to reach a final volume of 25 µl. Amplification was performed in a thermal cycler (Eppendorf, Germany) using the following program: initial denaturation at 96°C for 5 min; followed by 35 cycles including 30 s at 94 °C, 30 *s* at 55 °C, and 1 min at 72 °C, followed by a final extension step at 72 °C *for 5 min*. The PCR reaction products along with a 100 bp DNA ladder were visualized by electrophoresis at 80 volts for one hour in a 1.5% agarose gel containing DNA green viewer dye and finally evaluated in a gel documentation system (Gholami et al., 2016, Pacheco et al., 2019).

**Table 1.** Sequence characteristics of primers used to identify qnr *and aac(6')-Ib* genes

| Gene | Primer type | Primer sequence | Product size (bp) | length (bp) |
|---|---|---|---|---|
| *aac(6')-Ib* | Forward<br>Reverse | 5′-TTGCGATGCTCTATGAGTGGCTA-3′<br>5′-CTCGAATGCCTGGCGTGTTT-3′ | 482 | 23<br>20 |
| *qnr A* | Forward<br>Reverse | 5′- ATTTCTCACGCCAGGATTTG -3′<br>5′- GATCGGCAAAGGTTAGGTCA -3′ | 516 | 20<br>20 |
| *qnr B* | Forward<br>Reverse | 5′- GATCGTGAAAGCCAGAAAGG -3′<br>5′- ATGAGCAACGATGCCTGGTA -3′ | 476 | 20<br>20 |
| *qnr S* | Forward<br>Reverse | 5′- GCAAGTTCATTGAACAGGGT -3′<br>5′- TCTAAACCGTCGAGTTCGGCG -3′ | 428 | 20<br>21 |

## 3. Results

The antibiotic susceptibility profile of the 73 isolates of *P. aeruginosa* is presented in Table 2. All isolates were resistant to nalidixic acid, levofloxacin, ofloxacin, and cefepime. Resistant rates to other antibiotics were 98.1% to imipenem, 97.2% to meropenem and ciprofloxacin (Fig 1). In this study, of 71 ciprofloxacin-resistant isolates, the MICs of 5 isolates were ≤ 8 µg/ml and 19 isolates were significantly inhibited at ≥ 32 µg/ml. The MIC of 47 isolates were between 8 to 32 µg/ml.

The results from PCR amplification products of the qnr *and aac (6')-Ib* genes among 73 *P. aeruginosa* isolates (Figure 2) showed that 8 isolates (11.0%) had at least one of these genes and 65 (89.0%) of the isolates did not have any resistant genes. The frequency of qnr *and aac (6')-Ib* genes in isolates of *P. aeruginosa* according to the type of gene was presented in Table 2.

Statistical analysis did not show a significant relationship between the presence of the qnr *and aac (6')-Ib* genes and resistance to ciprofloxacin, levofloxacin, and ofloxacin (P<0.01), but all isolates with one of these plasmid coding genes showed resistance to quinolone antibiotics (P<0.01). Unlike very high resistance of the isolates to these antibiotics which was detected by disc diffusion method, but in a small number of them qnr *and aac (6')-Ib* resistance genes were present. Therefore, it can be concluded that there are other mechanisms responsible for the development of quinolone resistance in *P. aeruginosa* strains.

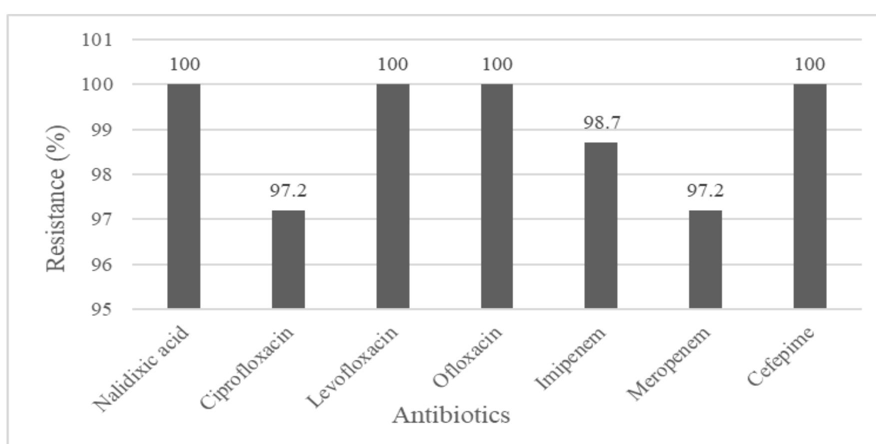



**Figure 1.** Antibiotic resistance profile of 73 *P. aeruginosa* strains isolated from burned patients

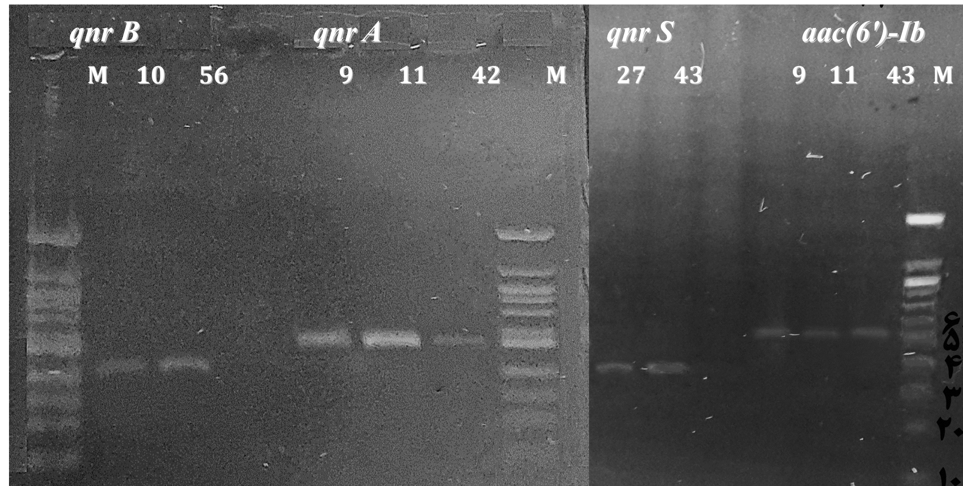



**Figure 2.** Agarose gel analysis of PCR amplification product using specific primers, M Marker

**Table 1.** Distribution of qnr and aac (6')-Ib genes in the isolates of *P. aeruginosa* from burn wounds

| Genes | No.(%) |
|---|---|
| *aac (6')-Ib* | - |
| qnrA | - |
| qnrB | 2(2.7) |
| qnrS | - |
| *aac (6')-Ib*+qnrA | 3(4.1) |
| *aac (6')-Ib*+qnrB | - |
| *aac (6')-Ib*+qnrS | 1(1.4) |
| qnrA+qnrB+qnrS | 1(1.4) |
| Total | 7(9.7) |

## 4. Discussion

The results of this study showed an increasing resistance to antibiotics in *P. aeruginosa* isolates causing burn infections among its potential to cause various other infections. Burns create a favorable environment for skin infections by removing the skin's defense barrier. Bacterial accumulation in burn wounds can be seen even with the use of topical antibacterial drugs (Kopecki, 2021). *P. aeruginosa* is one of the most important causes of nosocomial infections in burn patients (Vitkauskienė et al., 2010). One of the most important features of *P. aeruginosa* is its resistance mechanisms to known antibiotics including aminoglycosides, quinolones, and β-lactams (Freitas et al., 2002, Sasaki et al., 2004). One of the major mechanisms of resistance that has been detected in *P. aeruginosa* is intrinsic resistance, which involves the expression of resistance genes responsible for decreased outer membrane permeability, the production of efflux pumps that discharge the antibiotics out of the cell, as well as the production of enzymes that inactivate antibiotics (Breidenstein et al., 2011).
The results obtained by detection of antibiotic resistance in *P. aeruginosa* isolates in the present study differ from the results of other researchers' studies. In the study of Babaeekhou et al., the resistance incidence of *P. aeruginosa* isolates to ceftazidime and imipenem were reported to be 78.9% and 51.4%, respectively (Babaeekhou et al., 2018). In a study conducted by Rahimzadeh et al., the resistance incidence to ceftazidime-Aavibactam was 8% (Rahimzadeh et al., 2020).

Fluoroquinolones are the most effective antibiotics against *P. aeruginosa* infections. In this study, almost all of the isolates of *P. aeruginosa* showed resistance to ciprofloxacin, levofloxacin, and ofloxacin by the disk diffusion method, although a high resistance to cephalosporins was observed in P. aeruginosa strains. According to the previous studies, 25-30% of *P. aeruginosa* isolates were resistant to ciprofloxacin. For instance, Ruiz-Garbajosa & Cantón reported that the incidence of antibiotic resistance. among P. aeruginosa isolates to imipenem, meropenem, ciprofloxacin, and levofloxacin in Spanish hospitals were 51.6, 77.4, 84.8, 71.4, and 68.4, respectively (Ruiz-Garbajosa et al., 2017). These results are consistent with the results obtained in the present study. Rajaei et al., reported resistance to cefixime (80%), nalidixic acid (75%), imipenem (25%) and ciprofloxacin (35%). They detected quinolone resistance genes, including the qnrA (16.66%), qnrB (13.33%), qnrS (11.66%) and, aac(6)-Ib-cr (8.33%) among the isolates. In the Rajaei et al. study, the qnrA gene had the highest incidence rates, and they stated that the abundance of the qnr gene in P. aeruginosa clinical isolates is very high (Rajaei et al., 2017).

Molapour et al. studied 149 isolates of *P. aeruginosa* isolated from burn wounds and concluded that all of them were resistant to quinolones according to the Kirby Bauer method, but not all of them had qnr genes. The gene aac (6)-Ib was identified in all of their isolates (Molapour et al., 2020). Poirel et al. identified aacA29a and aacA29b as two aminoglycoside resistance genes in *P. aeruginosa* clinical isolates, which located at the two ends of the carbapenem-hydrolyzing β-lactamase gene cassette in class I integrons (Poirel et al., 2001).

Khan et al. investigated fluoroquinolone resistance genes (crpP and qnrVC1) in 33 strains of *P. aeruginosa* isolated from the cornea of keratitis patients in India. They reported that the presence of the crpP gene was not the only cause of fluoroquinolone resistance because a large number of selected isolates lacked this gene (Khan et al., 2020). The qnr genes which commonly located on mobile genetic elements, are major elements for the transferring resistance determinants against fluoroquinolones among clinical bacterial strains (Juraschek et al., 2021). AL-Marjani revealed that clinical isolates of P. aeruginosa showed higher resistance than environmental isolate. He detected the qnrS and, qnrA genes respectively, in 21.0% and 13.1% of clinical isolates of P. aeruginosa, although only one environmental isolate (1.3%) had the qnrS gene (AL-Marjani, 2014). Vaziri et al. detected the aac(6')-Ib-cr, and qnrB genes, respectively, in 55.6% and 34.9% of extended-spectrum beta-lactamase (ESBL)-producing Klebsiella pneumonia strains isolated from burn wounds of patients in Iran (Vaziri et al., 2020). El-Badawy et al. reported 92 clinical isolates of *Pseudomonas,* of which 42.4% were resistant to quinolones. They found qnrD, qnrS, and aac (6´)-Ib-cr plasmids in 79.5%, 79.5%, and 71.8% of the 39 isolates, respectively, while all three genes together were found in 56.4% of isolates. The qnrA and qnrB genes were not detected in any of the isolates (El-Badawy et al., 2019). Yang et al. investigated the molecular characterization of the resistance of P. aeruginosa isolates from South China to fluoroquinolones. The most studied strains were sensitive to polymyxin B, piperacillin, piperacillin/tazobactam, ceftazidime, and amikacin, although 65 isolates were detected that were resistant to ciprofloxacin. More ever, the resistant strains carried gyrA, gyrB and parC genes with at least one mutation although mutations were not detected in the parE gene. The qnrA1 gene was detected with low association levels to ciprofloxacin resistance in clinical P. aeruginosa isolates in their study (Yang et al., 2015).

A comparison of the results obtained in various studies as well as the present study shows that the incidence of resistance to fluoroquinolones varies in different parts of the world, which would be according to the pattern of the usage of these antibiotics, although it can be concluded that the prevalence of fluoroquinolone-resistant strains is increasing in all areas. New therapeutic strategies, including the development of novel antibiotics or alternative treatments for *P. aeruginosa* infections, are definitely required, especially for burned patients whose infections are resistant to conventional antibiotics.

## Conclusions

Antibiotic resistance varies according to treatment patterns in different regions. The results of this study showed high resistance to fluoroquinolone antibiotics such as Ciprofloxacin, Levofloxacin, and Ofloxacin among P. aeruginosa strains in burn infections, although the prevalence of fluoroquinolone-resistance genes was low. Because different strains of *P. aeruginosa* play an important role in the infection of burn wounds, it is necessary to implement an active monitoring system to detect the resistant bacterial strains in the hospital's burn wards.

## Refereces

AL-Marjani M. (2014). Presence of qnr gene in environmental and clinical Pseudomonas aeruginosa isolates in Baghdad. Int J Curr Microbiol App Sci. 3(7): 853-857 .

Alhussain F, Yenugadhati N, Al Eidan F, Al Johani S, Badri M. (2021). Risk factors, antimicrobial susceptibility pattern and patient outcomes of Pseudomonas aeruginosa infection: A matched case-control study. J Infect Public Health. 14(1): 152-157. https://doi.org/10.1016/j.jiph.2020.11.010

Babaeekhou L, Karshenasan H, Pishkar L. (2018). Antibiotic resistance in clinical isolates of Pseudomonas aeruginosa: A new viewpoint for antibiotic prescription. Avicenna J Clin Microbiol Infect. 5(3): 55-60. https://doi.org/10.34172/ajcmi.2018.11

Balouiri M, Sadiki M, Ibnsouda SK. (2016). Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 6(2): 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Barati M, Gholipourmalekabadi M, Ranjbari J, Shakib P, Bahar MA, Samadikuchaksaraei A. (2021). Antibiotic resistance pattern and molecular typing by PCR-RAPD analysis in clinical isolates of Pseudomonas aeruginosa from Motahari hospital, Tehran, Iran. J Kerman Univers Med Sci. 28(4): 420-426. https://doi.org/10.22062/jkmu.2021.91725

Bonev B, Hooper J, Parisot J. (2008). Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. Journal of Antimicrobial Chemotherapy. 61(6): 1295-1301. https://doi.org/10.1093/jac/dkn090

Breidenstein EB, de la Fuente-Núñez C, Hancock RE. (2011). Pseudomonas aeruginosa: all roads lead to resistance. Trends Microbiol. 19(8): 419-426. https://doi.org/10.1016/j.tim.2011.04.005

El-Badawy MF, Alrobaian MM, Shohayeb MM, Abdelwahab SF. (2019). Investigation of six plasmid-mediated quinolone resistance genes among clinical isolates of Pseudomonas: a genotypic study in Saudi Arabia. Infect Drug Resist. 12915-923. https://doi.org/10.2147/idr.S203288

Freitas AL, Barth AL. (2002). Antibiotic resistance and molecular typing of Pseudomonas aeruginosa: focus on imipenem. Braz J Infect Dis. 6(1): 1-7 .

Gholami A, Majidpour A, Talebi-Taher M, Boustanshenas M, Adabi M. (2016). PCR-based assay for the rapid and precise distinction of Pseudomonas aeruginosa from other Pseudomonas species recovered from burns patients. J Prev Med Hyg. 57(2): E-۸۱E85 .

Juraschek K, Deneke C, Schmoger S, Grobbel M, Malorny B, Käsbohrer A, Schwarz S, Meemken D, Hammerl JA. (2021). Phenotypic and genotypic properties of fluoroquinolone-resistant, qnr-carrying Escherichia coli isolated from the German food chain in 2017 .Microorganisms. 9(6): 1308. https://doi.org/10.3390/ microorganisms9061308

Kao CY, Wu HM, Lin WH, Tseng CC, Yan JJ, Wang MC, Teng CH, Wu JJ. (2016). Plasmid-mediated quinolone resistance determinants in quinolone-resistant Escherichia coli isolated from patients with bacteremia in a university hospital in Taiwan, 2001–2015. Scientific Reports. 632281. https://doi.org/10.1038/srep 32281. https://www.nature.com/articles /srep32281#supplementary-information

Khan M, Summers S, Rice SA, Stapleton F, Willcox MD, Subedi D. (2020). Acquired fluoroquinolone resistance genes in corneal isolates of Pseudomonas aeruginosa. Infect Genet Evol. 85104574. https://doi.org/10.1101/2020.05.17.10039 6

Koneman E, Allen S.2006. Color atlas and textbook of diagnostic microbiology, Lippincott Williams & Wilkins.

Kopecki Z. (2021). Development of next-generation antimicrobial hydrogel dressing to combat burn wound infection. Biosci Rep. 41(2). https://doi.org/10.1042/bsr20203404

Molapour A, Peymani A, Saffarain P, Habibollah-Pourzereshki N, Rashvand P. (2020). Plasmid-mediated quinolone resistance in Pseudomonas aeruginosa isolated from burn patients in Tehran, Iran. Infect Disord Drug Targets. 20(1): 49-55. https://doi.org/10.2174/ 1871526519666190206205521

Nahon C, Lehman D, Manuselis G.2015. Textbook of Diagnostic Microbiology, Saundera Elsevier.

Pacheco T, Bustos-Cruz RH, Abril D, Arias S, Uribe L, Rincón J, García J-C, Escobar-Perez J. (2019). Pseudomonas aeruginosa coharboring BlaKPC-2 and BlaVIM-2 carbapenemase genes. Antibiotics. 8(3): 98. https://doi.org/10.3390/antibiotics 8030098

Poirel L, Lambert T, Türkoglü S, Ronco E, Gaillard J, Nordmann P. (2001). Characterization of class 1 integrons from Pseudomonas aeruginosa that contain the bla(VIM-2) carbapenem-hydrolyzing beta-lactamase gene and of two novel aminoglycoside resistance gene cassettes. Antimicrob Agents Chemother. 45(2): 546-552.https://doi.org/10.1128/ AAC.45.2.546-552.2001

Rahimzadeh M, Habibi M, Bouzari S, Asadi Karam MR. (2020). First study of antimicrobial activity of ceftazidime-avibactam and ceftolozane-tazobactam against Pseudomonas aeruginosa isolated from patients with urinary tract infection in Tehran, Iran. Infect Drug Resist. 13533-541. https://doi.org/10.2147 /idr. S243301

Rajaei S, Kazemi-Pour N ,Rokhbakhsh-Zamin F. (2017). Frequency of plasmid-mediated quinolone resistance genes among clinical isolates of Pseudomonas aeruginosa in Kerman, Iran. Iran J Med Microbiol. 11(3): 10-18 .

Ruiz-Garbajosa P, Cantón R. (2017). Epidemiology of antibiotic resistance in Pseudomonas aeruginosa. Implications for empiric and definitive therapy. Rev Esp Quimioter. 30 Suppl 18-12 .

Sasaki M, Hiyama E, Takesue Y, Kodaira M, Sueda T, Yokoyama T. (2004). Clinical surveillance of surgical imipenem-resistant Pseudomonas aeruginosa infection in a Japanese hospital. J Hosp Infect. 56(2): 111-118. https://doi.org/10.1016/j.jhin.2003.10.020

Theodorou P, Thamm OC, Perbix W, Phan VTQ. (2013). Pseudomonas aeruginosa Bacteremia After Burn Injury: The Impact of Multiple-Drug Resistance. J Burn Care Res. 34(6): 649-658. https://doi.org /10.1097/BCR.0b013e318280e2c7

van Langeveld I, Gagnon RC, Conrad PF, Gamelli RL, Martin B, Choudhry MA, Mosier MJ. (2017). Multiple-drug resistance in burn patients: A retrospective study on the impact of antibiotic resistance on survival and length of stay. J Burn Care Res. 38(2): 99-105. https://doi.org/10.1097/BCR. 0000000000000479

Vaziri S, Afsharian M, Mansouri F, Azizi M, Nouri F, Madadi-Goli N, et al. (2020). Frequency of qnr and aac(6')Ib-cr genes among ESBL-producing Klebsiella pneumoniae strains isolated from burn patients in Kermanshah, Iran. Jundishapur J Microbiol. 13(7): e100348. https://doi.org/10.5812/jjm.100348.

Vitkauskienė A, Skrodenienė E, Dambrauskienė A, Macas A, Sakalauskas R. (2010). Pseudomonas aeruginosa bacteremia: resistance to antibiotics, risk factors, and patient mortality. Medicina 46(7): 490-495 .

Yang X, Xing B, Liang C, Ye Z, Zhang Y. (2015). Prevalence and fluoroquinolone resistance of Pseudomonas aeruginosa in a hospital of South China. Int J Clin Exp Med. 8(1): 1386-1390 .