

The pattern of antibiotic resistance and distribution of the biofilm-producing *Pseudomonas aeruginosa* (PeID, PslB) isolated from infectious hospital departments

SAGE Open Medicine

Volume 12: 1–10

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DOI: 10.1177/20503121241298826

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Abstract

Background: The ability of *Pseudomonas aeruginosa* to produce biofilm has established it as one of the most significant pathogens. The purpose of this study was to evaluate antimicrobial resistance and conduct a molecular investigation of the virulence genes *PslB* and *PeID* in *Pseudomonas aeruginosa* species isolated from patients.

Methodology: One hundred clinical isolates were collected from patients of different age groups who were hospitalized in Kermanshah and Sonqor hospitals. The isolates were obtained through culture on specific media, biochemical confirmatory tests, and gram staining for confirmation. Biofilm production was assessed using an indirect quantification method with crystal violet. Additionally, antibiotic resistance was determined through the disc various method following Clinical and Laboratory Standards Institute guidelines. Finally, the presence of genes related to *PslB* and *PeID* in resistant strains was examined using The polymerase chain reaction (PCR).

Results: The results indicate that the highest resistance and lowest sensitivity were related to nitrofurantoin 100 µg, while the lowest resistance and highest sensitivity were related to cefepime 30 mg. Biofilm phenotypes were categorized as weak in 7% ($n=7$) of isolates, medium in 13% ($n=13$), and high in 80% ($n=80$). The *PslB* and *PeID* genes were identified in 86% ($n=86$) and 38% ($n=38$) of isolates, respectively, while 4% ($n=4$) did not possess either of these two genes. Additionally, a majority of the isolates exhibited multidrug-resistance (87%) due to their moderate-to-high biofilm formation.

Conclusion: All isolates were capable of producing biofilm. A significant association were between strains with the high biofilm and multidrug-resistance species ($p < 0.05$). Multidrug-resistance (78%) isolates included 28% ($n=28$) of isolates that were *PslB+* *PeID+*, 45% ($n=45$) of isolates that were only *PslB+*, and 5 ($n=5$) isolates that were only *PeID+*. A significant relationship was found between the presence of the *PslB* gene multidrug-resistance and high producer ($p < 0.05$).

Keywords

P. aeruginosa, antibiotic resistance, *PslB*, *PeID*, biofilm, MDR

Date received: 13 August 2024; accepted: 23 October 2024

Introduction

As one of the most common nosocomial pathogens, *Pseudomonas aeruginosa* (*P. aeruginosa*) is involved in many severe opportunistic infections, especially in immunocompromised individuals.¹ This poses a pervasive problem as it complicates antibiotic treatments against *P. aeruginosa* due to the development of multidrug-resistant (MDR) species.^{2–4} The ability of *P. aeruginosa* to produce biofilm has placed this bacterium in the category of bacteria with chronic and complex infections. These bacteria protect themselves by producing biofilms, which include consisting of proteins, extracellular DNA, and exopolysaccharides. Biofilm create a

microbial complex that naturally poses many problems for antibacterial treatments.⁵

P. aeruginosa thrives in various aquatic environments and can cause both immediate and persistent infections in these settings. Bacterial conditions and host defense mechanisms

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impose different stresses on bacteria, forcing them to adapt in order to survive in diverse environments. The bacterium sustains itself by creating an extracellular polysaccharide enclosure around itself and is capable of causing both acute and chronic infections. Alginate, Psl, and Pel are three membrane polysaccharides secreted by *P. aeruginosa* that differ in their chemical structure features and biosynthetic mechanisms.⁶ Research on alginate, which is responsible for chronic lung infections, has helped researchers identify its production pathway. However, only a limited number of *P. aeruginosa* strains produce alginate, while most pathogenic strains produce Pel and Psl polysaccharides. The structural and functional similarity of alginate and bridge protein is significant.^{7,8} Biosynthesis of Psl involves the aggregation of pentameric Psl subunits linked to an isoprenoid lipid carrier, a process also observed in the *Escherichia coli* (*E. coli*) extracellular polysaccharides (EPS)/capsular polysaccharides (CPS) capsular biosynthesis pathway.⁶⁻⁹

Psl is classified as a neutral polysaccharide. Its structure consists of 5 units of D-mannose followed by D-mannose-glucose and finally a L-rhamnose. Psl plays a crucial role in interactions between bacterial cells during biofilm formation, making it important in the process of biofilm formation and maintenance.¹⁰ The geneset that produces this membrane polysaccharide is an operon containing 15 genes (*pslA* to *pslO*) that are transcribed and then translated as a polycistronic mRNA. An enzyme from the Psl operon is involved in making sugar-nucleotide precursors of Psl monosaccharide subunits, namely, D-mannose, L-rhamnose, and D-glucose. The PslB protein has GMP at the N-terminus and PMI at the C-terminus, giving it two distinct properties and functions. The PslB enzyme performs the first and third steps phosphomannose isomerase (PMI)/GDP-mannose pyrophosphorylase (GMP) guanosine diphosphate mannose (GDP-mannose) of converting fructose-6-phosphate to.¹¹

The Pel operon is involved in the formation of a pellicle, which is a layer of polymer/cells at the air-liquid interface of *P. aeruginosa* standing culture.¹² Structurally, exopolysaccharide P consists of 1→4 acetylated glucosamine sugar subunits and glucosamine sugars, and is sensitive to cellulose.¹³ The pel operon contains seven genes (*pelA* to *pelG*).¹⁴ Considering the potential of biofilm in increasing antibacterial resistance and prolonging infections caused by *P. aeruginosa*, identifying isolates with such a factor helps us to better understand the pathogenesis of the organism. Colvin et al.¹⁵ showed that pel exopolysaccharide has a structural and protective function in the biofilm matrix. Yang et al.'s¹⁶ findings also showed that psl plays a role in the antibiotic resistance of *P. aeruginosa* biofilms. Singkham et al.¹⁷ demonstrated the function of biofilms by decrease several genes, including biofilm synthesis genes (*algD* and *PslB*) and biofilm regulators (*gDsia*, *U*, *Ag*).

A study conducted in 2022 by Divyashree et al.¹⁸ focused on biofilm-related genes of *Psl* and *Pel* isolates. The study revealed that ampicillin (88.24%), nalidixic acid (83.82%), and

nitrofurantoin (64.71%) had the highest resistance rates. Also, the most dominant gene identified was *PslB*, with most isolates showing resistance to multiple drugs due to moderate-to-high biofilm formation.

This research aims to evaluate the pattern of antimicrobial resistance and the frequency of *PelD* and *PslB* genes in biofilm-producing *P. aeruginosa* isolated from hospital infectious departments. To the best of our knowledge, there has been little research conducted on the genes *PslB* and *PelD* in Iran. This type of study could provide valuable insights for the development and improvement of new and powerful antibiotics that target specific genes or products of combinations of genes involved in the formation of biofilms and the spread of MDR strains.

Methods

Sample collection

This cross-sectional study was conducted in 2024. After obtaining approval from the ethics committee and written informed consent from the legally authorized representative of under 18 years subjects, 100 clinical samples were collected from three different areas of Kermanshah and Sonqor hospitals over a period of 4 months. The samples included urine, bed sores, blood, lung secretions and burn wounds. Diagnostic tests were then performed on the samples. The study included patients of different age groups with at least a positive culture from the target organ, while patients with secondary infections of other organs were excluded from the study.

Phenotypical and biochemical confirmatory tests

All samples were cultured on cetrimide agar medium and then incubated for 24 h at 37°C. The presence of *P. aeruginosa* in the collected samples was indicated by a green pigment on the cetrimide agar grown on cetrimide agar medium at 42°C. The strains of *E. coli* ATCC- 29212 (colorless colony in cetrimide agar) and *P. aeruginosa* ATCC- 9037 were used as negative and positive controls, respectively, for detecting isolates of *P. aeruginosa*. Biochemical confirmatory tests were then performed on colonies grown on cetrimide agar medium. Isolates that were oxidase positive, hydrogen sulfide (H₂S)-negative, triple Sugar Iron (TSI)-positive, catalase-positive, citrate-positive, sulphide indole motility (SIM)-positive, urea-positive, oxidative-fermentative (OF)-positive, able to grow at 42°C, methyl red (MR)-positive and Voges-Proskauer (VP)-negative were selected.

Antibacterial susceptibility tests

Based on the recommendation of the Clinical and Laboratory Standards Institute, specific discs were selected and placement of two Muller Hinton Agar mediums (MERCK., Germany) with a concentration of half McFarland at a

Table 1. Primers sequences of *PslB* and *PelD* genes, as well as characteristics of their PCR products.

Gene	Sequence of primers	PCR products	References
<i>PslB</i>	F: 5' – GCTTCAAGATCAAGCGCATC – 3' R: 5' – ACCTCGATCATCACCAGGTC – 3'	160	20
<i>PelD</i>	F: 5' – AAGTCAGCGGCAACAACAC – 3' R: 5' – CGTTTCTCCCAGTACCTCAA – 3'	208	21

distance of at least 2.5 cm apart was made. The antibiotic sensitivity test was conducted by the agar disk diffusion method.

Antibacterial susceptibility was determined by disc diffusion using the following antibiotic discs (MAST, UK): imipenem (IMI 30 mg), gentamicin (GM 10 mg), nitrofurantoin (NIT 100 µg), ciprofloxacin (CIP 5 mg), cefepime (CEF 30 mg) and calactin (CL 10 mg).³

Biofilm formation assay

The microtitre-plate method³ along with an indirect quantification method with crystal violet was used to study *P. aeruginosa* biofilm formation. Initially, trypticase soy agar culture medium and a liquid solution containing 2% glucose were prepared. Bacterial samples were streaked on trypticase soy agar with 2% glucose and then incubated at 37°C for 20 h. The resulting colonies were collected with a loop and transferred to trypticase culture medium with 2% glucose to create a bacterial suspension equivalent to McFarland half turbidity. To prevent testing errors, the turbidity of the samples was measured visually and with a spectrophotometer at a wavelength of 625 nm, and their OD was observed in the numerical range of 0.08–0.1. Next, 200 µL of bacterial suspension were taken and aliquoted into a 96-well microtiter plate, which was then incubated for 24 h at 37°C. After incubation, all the wells of the plate were aspirated and washed with sterile physiological saline to remove any unattached cells. The attached bacteria were fixed with absolute methanol for 10 min, and then the plate was stained with crystal violet (1%). After a few seconds, the excess stain was removed by washing the plate with sterile distilled water. Any remaining stain was washed off with 200 µL of glacial acetic acid (33%v/v), and the OD was read with an enzyme-linked immunosorbent assay (ELISA) reader (Stat Fax 2100, Awareness Tech Inc., USA) at 630 nm (Stat Fax 2100, Awareness Tech Inc., USA).³ This test was repeated three times for each bacterial sample, with a well containing an empty culture medium that is the same as the trypticase the broth containing 2% glucose was considered as a blank.¹⁹ The standard strain of *P. aeruginosa* PAO1 ATCC (American Type Culture Collection) 27853 were used as positive control for biofilm production ATCC 15692.

Molecular study

The genomes of all isolates were extracted using the boiling method. Previously described primers were used to confirm

Table 2. Frequency of isolated patients according to age.

Age	N (%)	Women (%)	Men (%)
6<	3 (3)	52 (52)	48 (48)
6–10	12 (12)		
10>	85 (85)		
Medium	29.88		
%	100		

the presence of the *PslB* and *PelD* genes (Table 1). The primers were manufactured by Cinna gene (Iran). *P. aeruginosa* strain PAO1 was used as positive control. The PCR assay was performed in a 25 µL volume using the specific primers and the following time and thermal program: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 55°C for 60 s, extension at 72°C for 60 s, and a final extension of 10 min at 72°C. This was carried out in a thermocycler device (Eppendorf Master cycler Gradient, Germany).

Statistical analysis

By SPSS version 19.0 statistical software (IBM® SPSS®) was conducted statistical analysis. Chi-square test (χ^2) and Fisher's exact test (in cases where the frequencies were less than or equal to 5) were applied to check the differences in allele frequency and genotype distribution between healthy controls and affected patients. Logistic regression analysis was performed to calculate the odds ratio (OR) with confidence interval (CI) 95% adjusted for age and sex.

Results

Characteristics of clinical isolates of *P. aeruginosa*

One hundred isolates were obtained from various clinical samples taken from patients of different age groups who were hospitalized in Sonqor and Kermanshah hospitals. These isolates were identified using culture, Gram staining, and diagnostic-differential tests.

Out of 100 samples examined, 48 belonged to men and 52 to women. A statistical analysis using Chi-square and Kramer correlation coefficients and agreement (Cohen's Kappa ($\kappa=0$)), showed no notable association between *P. aeruginosa* infection and the gender of the patients. The

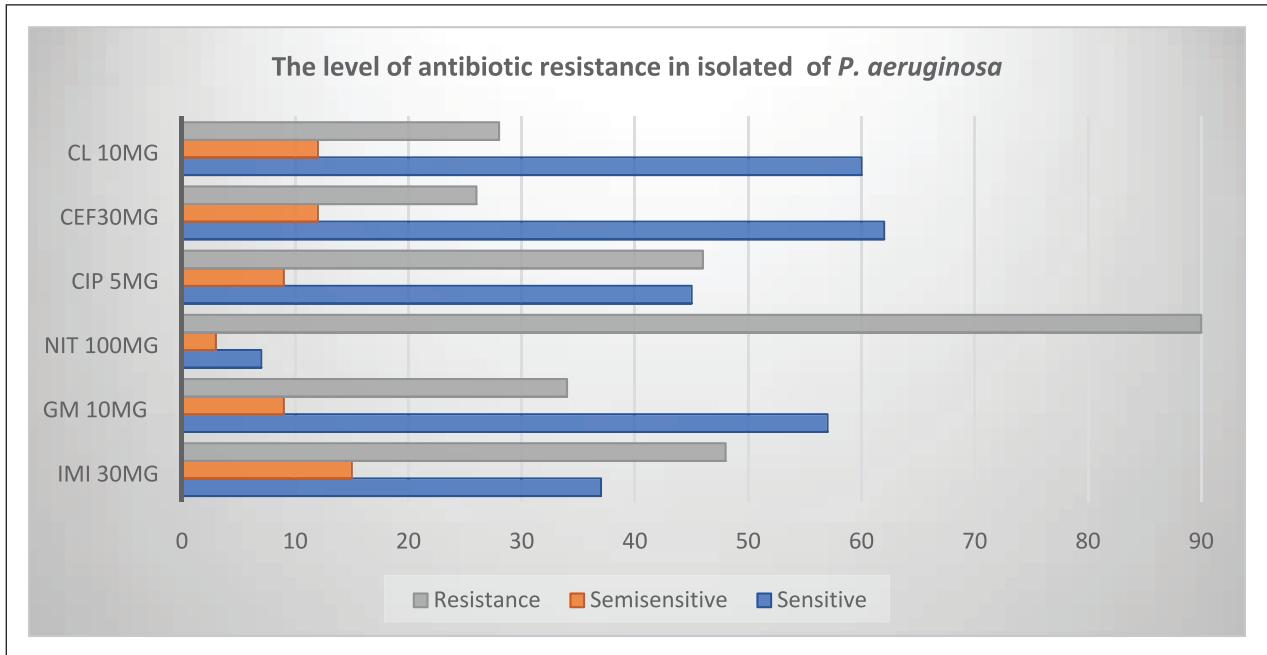


Figure 1. The level of antibiotic resistance in the 100 investigated *P. aeruginosa* isolates. The results indicate that the highest resistance and lowest sensitivity were related to NIT 100 µg, while the lowest resistance and highest sensitivity were related to CEF 30 mg.

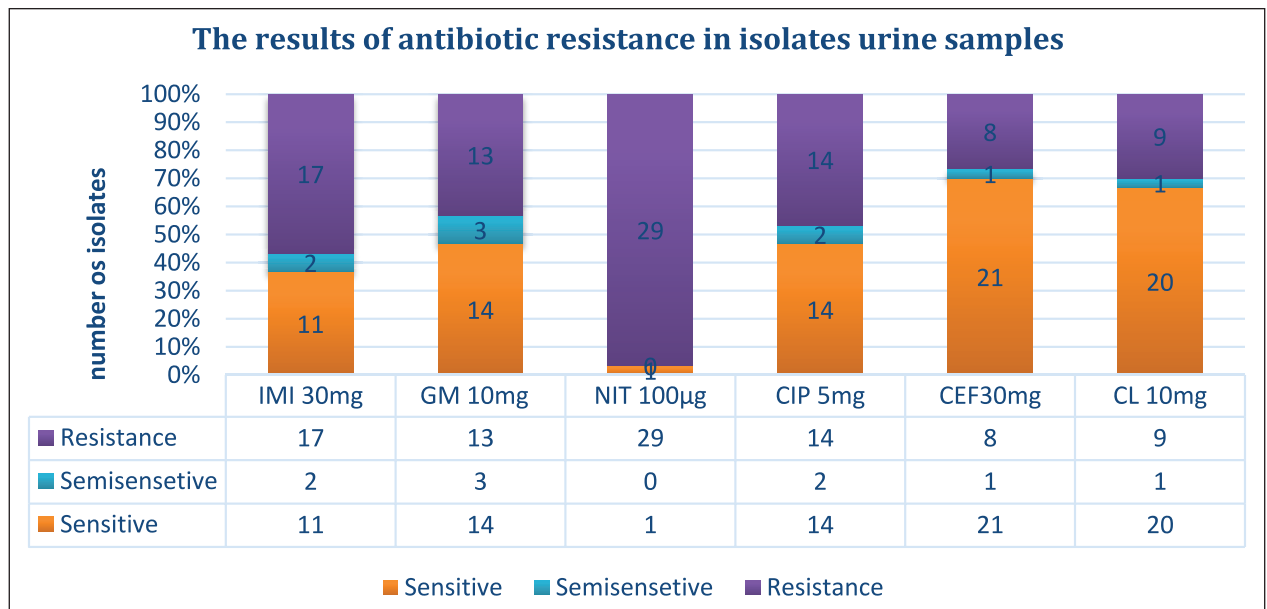


Figure 2. The level of antibiotic resistance of isolates related to urine samples.

age distribution of the patients was as follows: three individuals were below 6 years old; 12 individuals were between 6 and 10 years old, and 85 individuals were older than 10 years old (Table 2). A significant relationship was found between *P. aeruginosa* infection and the age of the patients ($p < 0.05$). The average age of the patients was 29.88 years. Out of 100 isolates, 30% ($n = 30$) were from urine, 10% ($n = 10$) from bed sores, 5% ($n = 5$) from blood, 32% ($n = 32$) from lung secretions, and 23% ($n = 23$) belonged to burn wounds.

Results of antibiogram test

The isolates demonstrated resistance to most of the commonly used antibiotics that were tested. Out of the 100 isolates, 90% of isolates showed maximum resistance to NIT 100 µg, and the lowest resistance was to CEF 30 mg (Figure 1).

In the examination of 30 urine isolates, it was observed that the highest antibiotic resistance was related to NIT 100 µg, while and the lowest was related to CEF 30 mg (Figure 2).

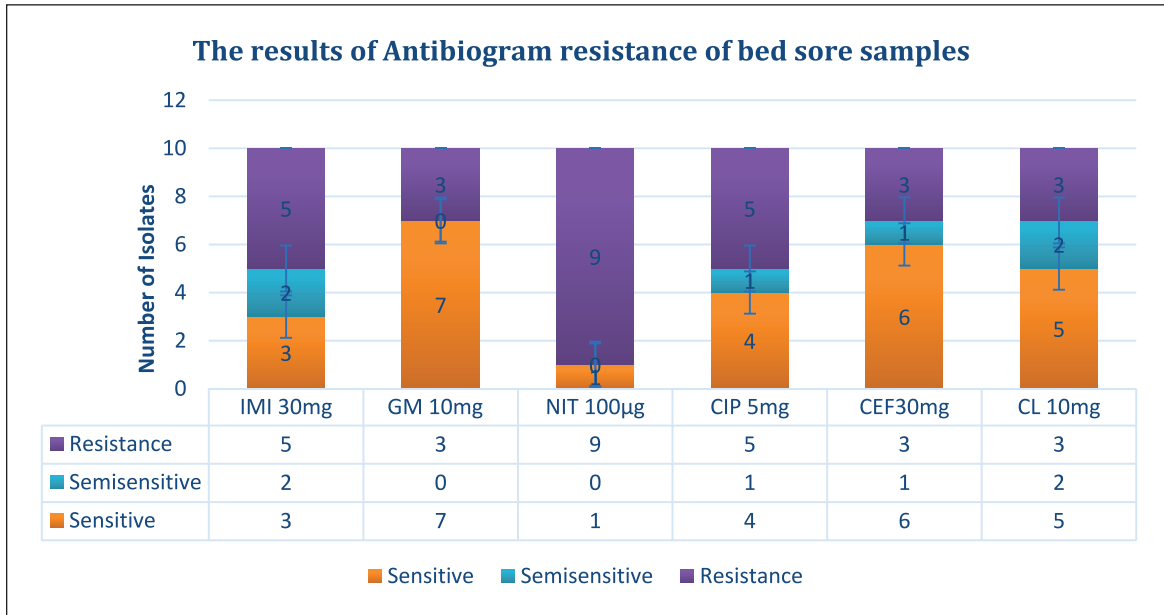


Figure 3. Antibigram test results of bed sore samples.

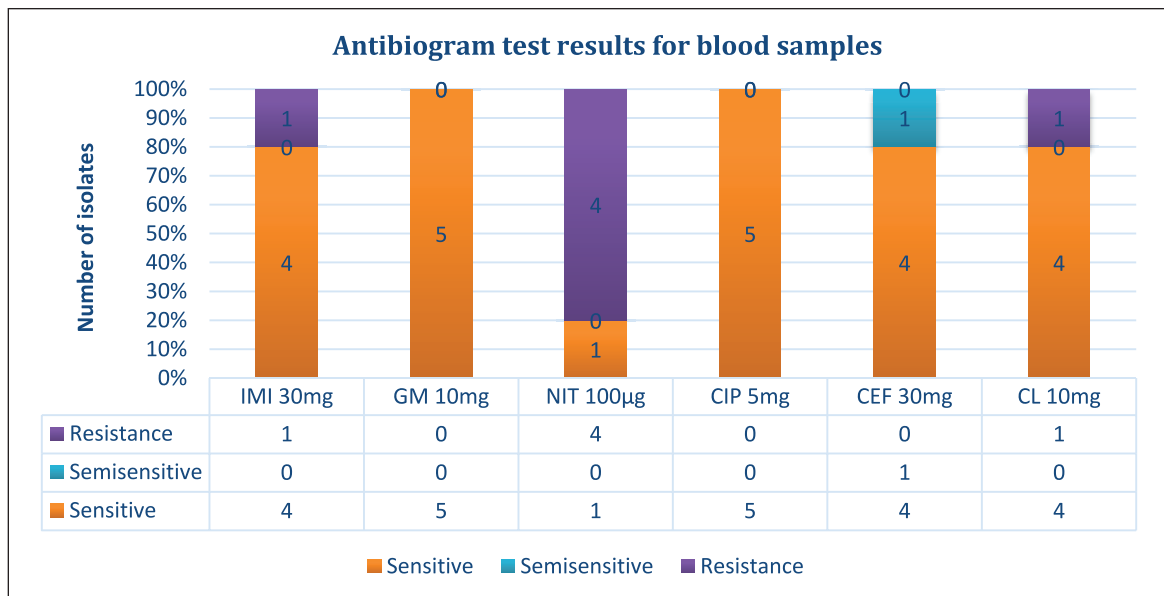


Figure 4. Antibigram test results of blood samples.

In 10 bedsore isolates, it was observed that the highest resistance was related to NIT 100 µg, while the lowest resistance was related to GM 10 mg, CEF 30 mg, and CL 10 mg, respectively. Among these samples, the most sensitive disc was GM 10 mg (Figure 3).

In five blood samples, the highest and lowest resistance was related to NIT 100 µg, while all samples were sensitive to GM 10 mg (Figure 4).

The results of the antibiogram test on 32 isolates isolated from pulmonary infections showed that in these samples, the highest resistance was to NIT 100 µg, and the lowest was to CEF 30 mg and CL 10 mg (Figure 5).

In burn wounds samples, the highest resistance was related to NIT 100 µg, and the lowest resistance was related to CEF 30 mg and CL 10 mg (Figure 6).

Results biofilm

Examining the results of biofilm formation by the collected isolates, it was found that averages optical absorbance of 100 isolates at the wavelength, with three repetitions, was higher than 0.18. This indicates that all isolates were biofilm. Results showed 7% of the strain showed weak biofilm formation (a16, a10, a9, a7, a6, a5, a4), while 13% the strains

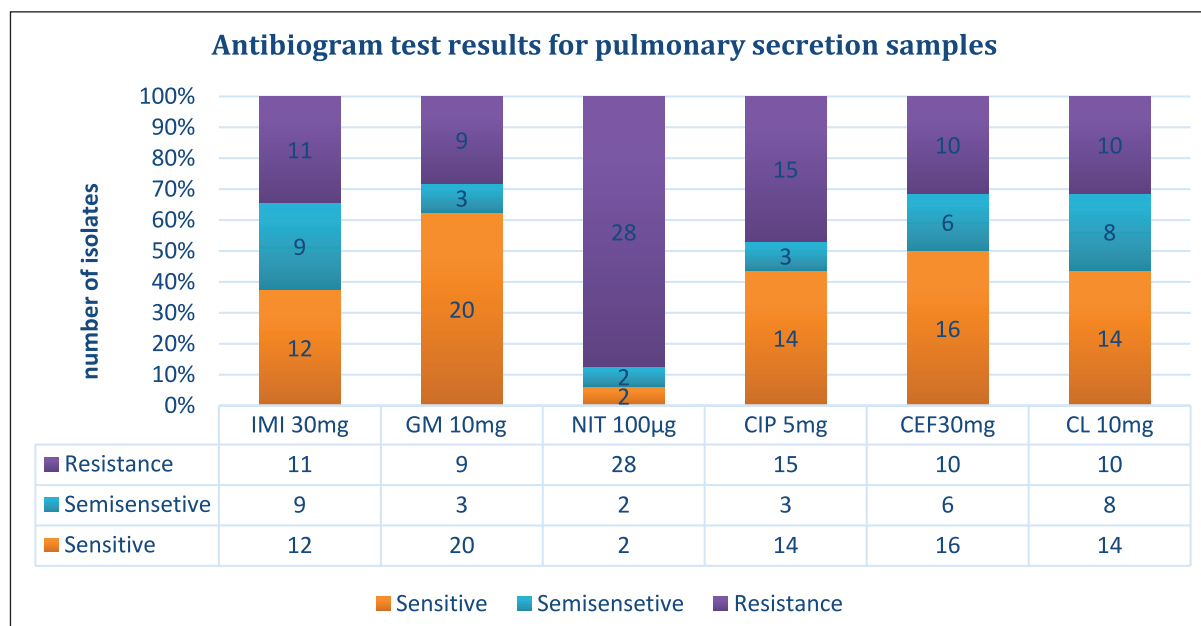


Figure 5. Antibiogram test results for pulmonary secretion samples.

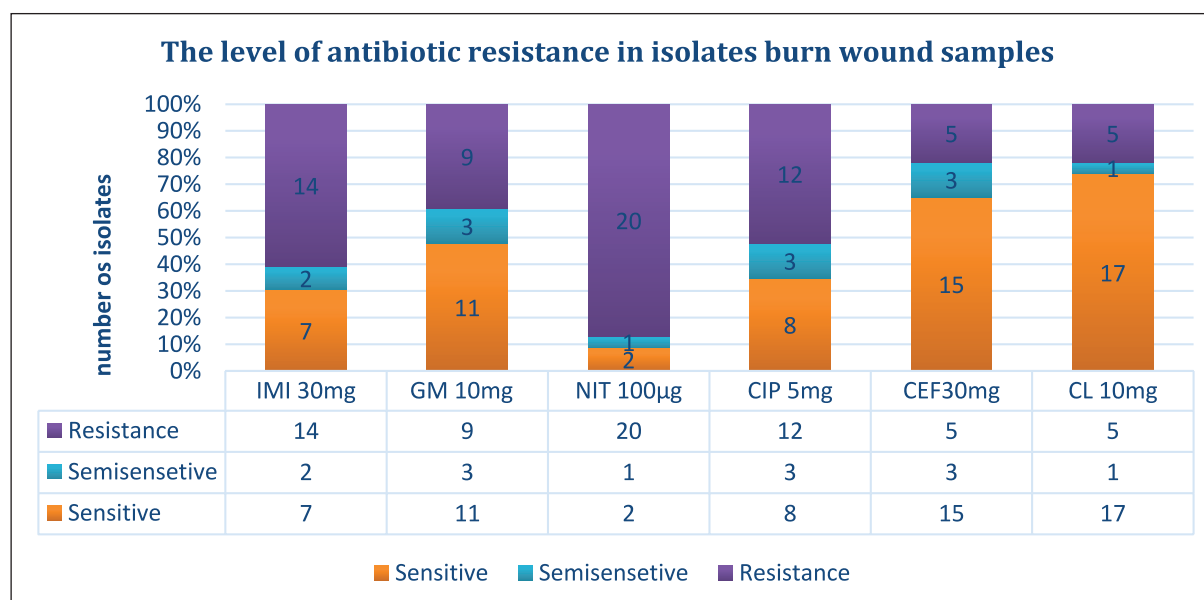


Figure 6. Antibiogram test results for burn wounds samples.

had medium biofilm formation (a28, a27, a25, a22, a19, a18, a17, a15, a14, a11, a8, a3, a2). The remaining 80% of the strains had strong biofilm formation.

Results of molecular tests

The *PslB* gene was found to have a frequency of 86% in isolates (Figure 7), while the *PelD* gene showed a frequency of 38% in isolates (Figure 8).

Discussion

The presence of *P. aeruginosa* strains resistant to antimicrobial substances in samples isolated from hospitals, surface water, and other environmental sources poses a serious threat to communities.¹⁷ *P. aeruginosa* creates conditions for opportunistic pathogens by producing biofilm-forming exopolysaccharides and forming biofilms.²⁰ Biofilm keeps bacteria together and helps them acquire antibiotic-resistant



Figure 7. Electrophoresis of PCR product for *PsIB* gene in the examined isolates. Column 1 on the right: positive control, column 2 of 50 pairs of ladders, column 3 of negative control, the rest of the cells are related to the isolates, all of which have a band of 160 bp.

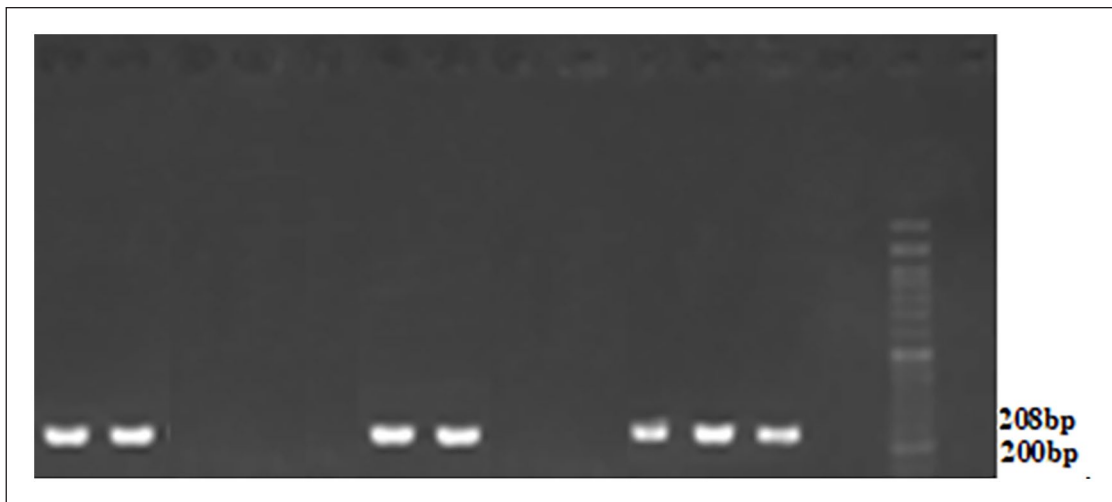


Figure 8. Electrophoresis of pcr product for *PeID* gene in investigated isolates. Column 1 on the right: positive control, column 2 is 50 pairs of ladders, column 3 is negative control, the rest of the cells are related to the isolates, all of which have a 200 bp band.

genes.²¹ Resistance of biofilm to antibiotics is a result of frequent exposure to high levels of antibiotics, as well as the repeated close contact of bacteria with these drugs. Additionally, genetic and physical characteristics of the bacterial strain, as well as physiological conditions, play a role in this resistance. Biofilm bacteria are resistant to antibiotic treatment due to internal and mutational resistance against antibiotics.²²

In this research, all *P. aeruginosa* isolates (100%) were capable of producing biofilms. We found a significant association between strains with the high biofilms and MDR species ($p < 0.05$). The phenotypic classification of biofilms identified in this study was as follows: weak (7%, $n = 7$); medium (13%, $n = 13$); and high (80%, $n = 80$). Jabalameli et al.²³ reported biofilm formation in more than 96% of the *P. aeruginosa* isolates collected from burn patients in Tehran.

In another study conducted at hospitals in Tabriz, Iran, a figure of 79% was reported for biofilm-forming clinical strains of *P. aeruginosa*.²⁴ Previously, Drenkard and Ausubel²⁵ reported that antibiotic-resistant strains of *P. aeruginosa* have a high ability to form biofilms.

Out of the 100 clinical strains tested for antibiogram, 48%, 34%, 90%, 46%, 26%, and 28% were resistant to IMI 30 mg, GM 10 mg, NIT 100 μ g, CIP 5 mg, CEF 30 mg, and CL 10 mg, respectively.

The results of the antibiogram test on urine ($n = 30$), bed sores ($n = 10$), blood ($n = 5$), pulmonary infections ($n = 32$), and burn wounds ($n = 23$) indicated that isolates have the highest resistance to NIT 100 μ g. Although the number of isolates differs in each group, it seems that NIT 100 is not appropriate for these isolates. Additionally, in urine, the lowest resistance was related to CEF 30 mg; in bed sores it was related to GM 10 mg,

CEF 30 mg, and CL 10 mg, with the most sensitive disc being GM 10 mg (Figure 3). In blood isolates all samples were sensitive to GM 10 mg (Figure 4). In pulmonary infections, the lowest resistance was to CEF 30 mg and CL 10 mg (Figure 5), and in burn wounds, the lowest resistance was related to CEF 30 mg and CL 10 mg (Figure 6). In another study, clinical isolates of *P. aeruginosa* exhibited high resistance to meropenem, ticarcillin, and other antibiotics.²⁶

All isolates were examined for the presence of the *PslB* and *PelD* genes. Among them, 86% of isolates ($n=86$) were positive for the *PslB* gene and 38% for the *PelD* gene. Additionally, 28% ($n=28$) of isolates had both genes (*PslB*⁺ *PelD*⁺). All of these were MDR and high producer biofilms. Meanwhile, 4% of isolates were negative for both genes (*PslB*⁻ *PelD*⁻) and were weak biofilm producer. One isolate from the *PelD*⁺ group was weak. The high producer biofilm group includes 28 (*PslB*⁺ *PelD*⁺), 52 *PslB*⁺ isolates, and 10 isolates with only *PelD*⁺. Among the *PelD*⁺ isolates, three were biofilm producers and seven were moderate biofilm producers. Six isolates from the only *PslB*⁺ isolates were moderate biofilm producers. Therefore, a significant relationship was found between the *PslB* gene MDR and high producer ($p < 0.05$). MDR (78%) isolates included 28% ($n=28$) of isolates that were *PslB*⁺ *PelD*⁺, 45% ($n=45$) of isolates that were only *PslB*⁺, and 5 ($n=5$) isolates that were only *PelD*⁺. A significant relationship was found between the presence of the *PslB* gene MDR and high producer ($p < 0.05$). Bavashe and Karmoustaji²⁷ reported that 27.8% of *P. aeruginosa* clinical isolates were MDR.

The main exopolysaccharides secreted by bacteria that are important in biofilm formation are Psl, Alg, and Pel.^{28,29} In nonmucoid bacterial strains of *P. aeruginosa*, exopolysaccharides Psl and Pel are essential for maintaining biofilm structure. Exopolysaccharide Pel contains a high amount of glucose, necessary for pellicle formation, and plays a structural and protective role in the biofilm matrix.¹⁵ On the other hand, Psl is involved in biofilm formation in both nonmucoid and mucoid strains of *P. aeruginosa*.^{30,31} Divyashree et al.¹⁷ found that *PslB* was the most dominant gene, and that most of the isolates were resistant to several drugs due to moderate and high biofilm formation.

In 2019, Whitfield et al.^{26,32} identified stable *PelDEFG* gene clusters over 500 diverse proteobacterial species. Using *P. aeruginosa*, as a model, they demonstrated that *PelD*, *PelE*, *PelF*, and *PelG* form a complex in the inner membrane and suggested that this complex represents a previously unidentified Pel polysaccharide synthase, responsible for the polymerization and translocation of Pel to the cytoplasmic membrane. In 2018, Li et al.³³ conducted a study on the effects of diallyl disulfide (DADS) on the growth, virulence factor production (elastase, pyocyanin, biofilm, and swarming motility), and essential gene expression of *P. aeruginosa* PAO1, specifically in relation to QS and virulence. The *lasB* gene, which encodes *lasB* elastase, is regulated by the *las*, *rhl*, and *pqs* systems, so the down-regulation of genes in these systems enhances the down-regulation of *lasB*.

Additionally, *phzM* (encoding pyocyanin), *pslB* (responsible for biofilm matrix polysaccharide production), and *chiC* (encoding chitinase) were positively activated by *LasR*. Downregulation of *lasR* transcription further downregulated *phzM* and *phzMC* transcription. A study conducted by Kamali et al.³⁴ in 2020 showed that isolates exhibited resistance to the antibiotic's amikacin, piperacillin/tazobactam, and levofloxacin. All three genes related to biofilm were present simultaneously in 87.5% of isolates, while 13.5% of isolates did not have any of the tested genes. From the results of this study, combination therapy including an anti-pseudomonad beta-lactam (piperacillin/tazobactam or ceftazidime) and an aminoglycoside or carbapenem (imipenem, meropenem) with fluoroquinolones along with an aminoglycoside can be used against *Pseudomonas*.

One of the shortcomings of this study was the small number of samples. Additionally, it is recommended to collect samples from hospital fluids simultaneously. Another limitation of the study was the small number of antibiotics that were examined, due to limited funding. We did not use a specific formula for calculating the sample size, which was a limitation of this study. Sampling was based on convenience due to the cost of experimentation, limited availability of samples, and time constraints. Additionally, we should expand our research by investigating other biofilm genes in the negative biofilm samples for these two genes. Next, we should investigate the positive samples of the two genes in this assay for other biofilm genes. Another objective is to select different antibiotics and test their effectiveness.

Conclusion

The public health implications of *P. aeruginosa* should not be underestimated as it remains a major cause of healthcare-associated infections in Asia and has limited treatment options. This study revealed that isolates of *P. aeruginosa* exhibit a high percentage of resistance to commonly used drugs against this microorganism. In this research, all *P. aeruginosa* isolates (100%) were capable of producing biofilm. We found a significant association between strains with high biofilm and MDR species ($p < 0.05$). Out of the 100 clinical strains tested for antibiogram, 90% of them were resistant to NIT 100 μ g and 26% were sensitive to CEF 30 mg. All isolates were examined for the presence of the *PslB* and *PelD* genes. Among them, 86% of isolates ($n=86$) were positive for the *PslB* gene and 38% for the *PelD* gene. Additionally, MDR (78%) isolates included 28% ($n=28$) of isolates that were *PslB*⁺ *PelD*⁺, 45% ($n=45$) of isolates that were only *PslB*⁺, and 5 ($n=5$) isolates that were only *PelD*⁺. A significant relationship was found between the presence of the *PslB* gene MDR and high producer ($p < 0.05$). This study highlights the role of biofilms as a mechanism for bacterial survival.

Acknowledgements

Thank you to everyone who assisted us in conducting this research.

Authors' contributions

FK and NM developed the concept and design of the study, collected, and analyzed the data. The first draft and revised of the article was prepared by all authors; FK the manuscript; All authors reviewed and approved the article.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Ethics approval

This research was ethically approved by the Ethics Committee of the Sanandaj Branch, Islamic Azad University, Sanandaj, Iran (IR. IAU.SDJ.REC.1403.071). Also, all protocols involving human subjects comply with the requirements of the Declaration of Helsinki of the Iranian Ministry of Health and Medical Education.

Informed consent

Written informed consent was obtained from the Legally Authorized Representative of under 18 years subjects.

Trial registration

Not applicable.

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References

1. Stover CK, Pham XQ, Erwin AL, et al. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* 2000; 406: 959–964.
2. O'Toole GA. Microtiter dish biofilm formation assay. *J Vis Exp* 2011; 47: 2437.
3. Noyal MJ, Menezes GA, Harish BN, et al. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative gram-negative bacteria. *Indian J Med* 2009; 129(6): 707–712.
4. Shariati A, Azimi T, Ardebili A, et al. Insertional inactivation of oprD in carbapenem-resistant *Pseudomonas aeruginosa* strains isolated from burn patients in Tehran, Iran. *New Microbes New Infect* 2018; 21: 75–80.
5. Ito CAS, Bail L, Arend LNVS, et al. The activity of ceftazidime/avibactam against carbapenem-resistant *Pseudomonas aeruginosa*. *Infect Dis* 2021; 53: 386–389.
6. Kalluf KO, Arend LN, Wuicik TE, et al. Molecular epidemiology of SPM-1-producing *Pseudomonas aeruginosa* by rep-PCR in hospitals in Parana, Brazil. *Infect Genet Evol* 2017; 49: 130–133.
7. Ramos GP, Rocha JL and Tuon FF. Seasonal humidity may influence *Pseudomonas aeruginosa* hospital-acquired infection rates. *Int J Infect Dis* 2013; 17: e757–e761.
8. Tuon FF, Gortz LW and Rocha JL. Risk factors for pan-resistant *Pseudomonas aeruginosa* bacteremia and the adequacy of antibiotic therapy. *Braz J Infect Dis* 2012; 16: 351–356.
9. Turkina MV and Vikström E. Bacteria-host crosstalk: sensing of the quorum in the context of *Pseudomonas aeruginosa* infections. *J Innate Immun* 2019; 11: 263–279.
10. Nathwani D, Raman G, Sulham K, et al. Clinical and economic consequences of hospital-acquired resistant and multidrug-resistant *Pseudomonas aeruginosa* infections: a systematic review and meta-analysis. *Antimicrob Resist Infect Control* 2014; 3: 32.
11. Zowawi HM, Harris PN, Roberts MJ, et al. The emerging threat of multidrug-resistant gram-negative bacteria in urology. *Nat Rev Urol* 2015; 12: 570–584.
12. Tuon FF, Cieslinski J, Rodrigues SDS, et al. Evaluation of in vitro activity of ceftolozane-tazobactam against recent clinical bacterial isolates from Brazil—The EM200 study. *Braz J Infect Dis* 2020; 24: 96–103.
13. Ito CAS, Bail L, Arend LNVS, et al. Evaluation of MicroScan WalkAway for determination of ceftazidime-avibactam and ceftolozane-tazobactam susceptibility in carbapenem-resistant gram-negative bacilli. *J Clin Microbiol* 2021; 59: e0153621.
14. Bail L, Ito CAS, Arend LNVS, et al. Activity of imipenem-relebactam and ceftolozane-tazobactam against carbapenem-resistant *Pseudomonas aeruginosa* and KPC-producing Enterobacterales. *Diagn Microbiol Infect Dis* 2021; 102: 115568.
15. Colvin KM, Irie Y, Tart CS, et al. The Pel and Psl polysaccharides provide *Pseudomonas aeruginosa* structural redundancy within the biofilm matrix. *Environ Microbiol* 2012; 14(8): 1913–1928.
16. Yang L, Rybtke MT, Jakobsen TH, et al. Computer-aided identification of recognized drugs as *Pseudomonas aeruginosa* quorum-sensing inhibitors. *Antimicrob Agents Chemother* 2009; 53: 2432–2443.
17. Singkham-In U, Thaveekarn W, Noiphrom J, et al. Hydrogen peroxide from L-amino acid oxidase of king cobra (*Ophiophagus hannah*) venom attenuates *Pseudomonas biofilms*. *Sci Rep* 2023; 13(1): 11304.
18. Divyashree M, Mani MK and Karunasagar I. Association of exopolysaccharide genes in biofilm developing antibiotic-resistant *Pseudomonas aeruginosa* from hospital wastewater. *J Water Health* 2022; 20(1): 176–184.
19. Dheepa M, Vinitha LR and Appalaraju B. Comparison of biofilm production and multiple drug resistance in clinical isolates of *Acinetobacter baumannii* from a tertiary care hospital in South India. *Int J Pharm Biomed Sci* 2011; 2(4): 103–107.
20. Jackson KD, Starkey M, Kremer S, et al. Identification of Psl, a locus encoding a potential exopolysaccharide that is essential for *Pseudomonas aeruginosa* PAO1 biofilm formation. *J Bacteriol* 2004; 186(14): 4466–4475.
21. Gupta P, Dash PK, Sanjay TD, et al. Cloning and molecular characterization of the phlD gene involved in the biosynthesis of “phloroglucinol”, a compound with antibiotic properties from plant growth promoting bacteria *Pseudomonas* spp. *Antibiotics (Basel)* 2023; 12(2): 260.
22. Goltermann L and Tolker-Nielsen T. Importance of the exopolysaccharide matrix in antimicrobial tolerance of *Pseudomonas aeruginosa* aggregates. *Antimicrob Agents Chemother* 2017; 61: e02696-16.

23. Jabalameli F, Mirsalehian A, Khoramian B, et al. Evaluation of biofilm production and characterization of genes encoding type III secretion system among *Pseudomonas aeruginosa* isolated from burn patients. *Burns* 2012; 38(8): 1192–1197.
24. Gottaslo R and Salahi B. Effects of oxygen on in-vitro biofilm formation and antimicrobial resistance of *Pseudomonas aeruginosa*. *Pharmaceut Sci* 2013; 19(3): 96–99.
25. Drenkard E and Ausubel FM. *Pseudomonas* biofilm formation and antibiotic resistance are linked to phenotypic variation. *Nature* 2002; 416(6882): 740–743.
26. Khan MA and Faiz A. Antimicrobial resistance patterns of *Pseudomonas aeruginosa* in tertiary care hospitals of Makkah and Jeddah. *Ann Saudi Med* 2016; 36(1): 23–28.
27. Bavasheh N and Karmostaji A. Antibiotic resistance pattern and evaluation of blaOXA-10, blaPER-1, blaVEB, blaSHV genes in clinical isolates of *Pseudomonas aeruginosa* isolated from hospital in south of Iran in 2014–2015. *Infect Epidemiol Med* 2017; 3(1): 1–5.
28. Stewart PS and Franklin MJ. Physiological heterogeneity in biofilms. *Nat Rev Microbiol* 2008; 6: 199–210.
29. Franklin MJ, Nivens DE, Weadge JT, et al. Biosynthesis of the *Pseudomonas aeruginosa* extracellular polysaccharides, alginate, Pel, and Psl. *Front Microbiol* 2011; 2: 167.
30. Leid JG, Willson CJ, Shirtliff ME, et al. The exopolysaccharide alginate protects *Pseudomonas aeruginosa* biofilm bacteria from IFN-gamma-mediated macrophage killing. *J Immunol* 2005; 175: 7512–7518.
31. Davis SC, Ricotti C, Cazzaniga A, et al. Microscopic and physiologic evidence for biofilm-associated wound colonization in vivo. *Wound Repair Regen* 2008; 16(1): 23–29.
32. Whitfield GB, Marmont LS, Ostaszewski A, et al. Pel polysaccharide biosynthesis requires an inner membrane complex comprised of PelD, PelE, PelF, and PelG. *J Bacteriol* 2020; 202(8): e00684-19.
33. Li WR, Zeng TH, Yao JW, et al. Diallyl disulfide from garlic oil inhibits *Pseudomonas aeruginosa* quorum sensing systems and corresponding virulence factors. *Microb Biotechnol* 2021; 14(2): 677–691.
34. Kamali E, Jamali A, Ardebili A, et al. Evaluation of antimicrobial resistance, biofilm forming potential, and the presence of biofilm-related genes among clinical isolates of *Pseudomonas aeruginosa*. *BMC Res Notes* 2020; 13(1): 1–6.