International Journal of Biomedicine 15(4) (2025) 727-730
http://dx.doi.org/10.21103/Article15(4)_OA13

ORIGINAL ARTICLE

Antimicrobial Resistance

INTERNATIONAL JOURNAL OF BIOMEDICINE

Biofilm-Associated Genes and Antibiotic Susceptibility in Burn-Isolated *Pseudomonas aeruginosa*

Fatimah A. Abdul Jabbar[1], Mustafa S. Al-Salmani[2], Hadeer R. Kamel[3], Hasan A. Aal Owaif[1*]

[1]*Department of Applied Biological Science, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq*
[2]*Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq*
[3]*College of Science, Al-Nahrain University, Baghdad, Iraq*

Abstract

Background: Burn injuries reduce skin protection and immune responses, making them a global health issue. Among the most prevalent opportunistic bacteria in burn wounds is *Pseudomonas aeruginosa*, which is drug-resistant and produces biofilms. This study investigated biofilm-associated virulence genes, antibiotic susceptibility, and the link between gene expression, biofilm production, and antibiotic resistance.

Methods and Results: Burn patients hospitalized in Baghdad between June and August 2024 provided 120 burn swabs. *P. aeruginosa* isolates were identified using biochemical tests and the VITEK-2 system. Susceptibility to antibiotics was determined using the Kirby-Bauer disk diffusion technique and interpreted in accordance with the 2024 CLSI criteria. A microtiter plate test was used to quantify the production of biofilm at an optical density (OD) of 570 nm. The *algD*, *pelA*, and *pslA* genes were detected by PCR. *P. aeruginosa* has been verified in 57 (47.5%) of the isolates. Of them, 91.2% were resistant to ceftazidime, 87.7% to imipenem, 73.7% to gentamicin, and 61.4% to ciprofloxacin. MDR was detected in 63.1% of isolates. In 49.1%, 35%, and 15.9% of the isolates, biofilm development was strong, moderate, and weak, respectively. The *algD*, *pelA*, and *pslA* genes were detected in 86.0%, 68.4%, and 59.6% of the isolates, respectively. A clear relationship was observed between these genes and biofilm production and resistance patterns.

Conclusion: The results in our study support a robust link between biofilm production, antibiotic resistance, and genes related to biofilm production by *P. aeruginosa* isolated from burn sites. Implementing gene-targeted techniques and optimal combination treatment may greatly enhance infection management and patient outcomes in burn care facilities.(**International Journal of Biomedicine. 2025;15(4):727-730.**)

Keywords: *Pseudomonas aeruginosa* • *b*urn infections • antibiotic susceptibility • biofilm production • virulence genes

For citation: Jabbar FAA, Al-Salmani MS, Kamel HR, Aal Owaif HA. Biofilm-Associated Genes and Antibiotic Susceptibility in Burn-Isolated *Pseudomonas aeruginosa*. International Journal of Biomedicine. 2025;15(4):727-730. doi:10.21103/Article15(4)_OA13

Abbreviations

CLSI, Clinical and Laboratory Standards Institute; **MDR**, multidrug resistance; **PCR**, polymerase chain reaction.

Introduction

Burn accidents are unfortunately very common and affect many individuals all over the world. Burns destroy the skin's protective barrier, leading to decreased local and systemic immune responses and creating an environment susceptible to microbial colonization and infection.[1,2] *P. aeruginosa* is known as a prevalent opportunistic bacterium responsible for severe burn wound infections, which are frequently linked with more extended hospitalization and higher morbidity.[3] This Gram-

negative bacterium demonstrates a wide range of virulence characteristics, including biofilm formation, production of exotoxins, and development of exoenzymes, which contribute to its persistence and toxicity in burn wounds.[4] *P. aeruginosa* biofilm development is a major pathogenicity mechanism, allowing the bacteria to cling securely to tissues and medical equipment while shielding themselves from host immune responses and antibiotic treatments.[5] The biofilm matrix hinders antibiotic penetration and protects bacterial cells, resulting in persistent infections that are difficult to eliminate.[6] Furthermore, *P. aeruginosa* strains generating biofilms typically exhibit multidrug resistance (MDR), limiting therapeutic choices and resulting in treatment failure.[7] Biofilm production and structural integrity depend heavily on the genetic regulating biofilm-related genes such *algD*, *pelA*, and *pslA*.[8] Higher expression of these genes corresponds with improved biofilm development and increased resistance to several medications.[9] Understanding the interactions between virulence gene expression, biofilm formation, and antibiotic sensitivity is critical for designing effective treatment methods for *P. aeruginosa* infections in burn patients. The purpose of this study was to investigate the antibiotic resistance profile, biofilm-forming potential, and expression of biofilm-associated genes in *P. aeruginosa* isolated from burn infections in hospitals in Baghdad.

Materials and Methods

Between June and August 2024, 120 burn swabs were collected from burn patients attending several hospitals in Baghdad. Conventional biochemical assays were used to identify *P. aeruginosa* isolates, which were then verified using the automated VITEK-2 system.[10] The Kirby-Bauer disk diffusion technique was used on Mueller-Hinton agar plates to determine antimicrobial susceptibility, and the data were interpreted in accordance with CLSI 2024 criteria.[11] The antibiotics examined were imipenem (10 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), colistin (10 µg), and amikacin (30 µg). Biofilm production was assessed using the microtiter plate assay with crystal violet staining, and optical density was evaluated at 570 nm using an ELISA reader.[6] The detection of biofilm-associated genes (*algD*, *pelA*, and *pslA)* was determined using PCR. The sequences of primers are given in Table 1.

Statistical analysis was performed using GraphPad Prism v. 8. Baseline characteristics were summarized as frequencies and percentages for categorical variables. Group comparisons were performed using the chi-square test. A *P*-value of <0.05 was considered statistically significant.

Table1.

PCR primer sequences for biofilm-related genes.[12]

Gene	Primer Sequence (5'–3')	Product size (bp)
algD	F: ATCGTCCAGCGACTACCTTC R: CGGTTGTCAGGTAGCCACTT	210
pelA	F: GCTGATGCGGTTCTTCTGTC R: CTGTTCGCCAGGAAAGTACC	195
pslA	F:TCGAGTGGAGAGACGAAGGA R: CTGGTGATCGCTGATGGTAG	182

Results

From 120 swabs, 57 (47.5%) *P. aeruginosa* isolates were identified. Antibiotic susceptibility testing revealed substantial resistance rates to imipenem (87.7%), ceftazidime (91.2%), and ciprofloxacin (61.4%), whereas resistance to colistin remained low (8.8%) (Table 2). Of the isolates, 63.1% exhibited multidrug resistance. The findings of the biofilm test categorized isolates as strong (49.1%), moderate (35%), and weak (15.9%). The *algD*, *pelA*, and *pslA* genes were detected in 86.0%, 68.4%, and 59.6% of the isolates, respectively. A substantial relationship was found between high production of biofilm and resistance to imipenem, ciprofloxacin, ceftazidime, and gentamicin (Table 3). In addition, *algD*, *pelA* and *pslA*-detected isolates had high biofilm formation in 55.1%, 56.4%, and 55.9%, respectively. (Table 4).

Table 2.

Antimicrobial susceptibility of P. aeruginosa isolates.

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Ceftazidime	5 (8.8)	0 (0)	52 (91.2)
Imipenem	7 (12.3)	0 (0)	50 (87.7)
Ciprofloxacin	22 (38.6)	0 (0)	35 (61.4)
Gentamicin	25 (43.8)	5 (8.8)	27 (73.7)
Colistin	52 (91.2)	0 (0)	5 (8.8)
Amikacin	20 (35.1)	7 (12.3)	30 (52.6)

Table 3.

Biofilm production and antibiotic resistance among P. aeruginosa isolates.

Antibiotic	High production (n=28)	Intermediate production (n=20)	Low production (n=9)
	n (%)	n (%)	n (%)
Ceftazidime	27 (96.4)	18 (90)	7 (77.8)
Imipenem	26 (92.8)	15 (75)	9 (100)
Ciprofloxacin	22 (78.6)	11 (55)	2 (22.2)
Gentamicin	20 (71.4)	10 (50)	4 (44.4)
Colistin	1 (3.6)	2 (10)	2 (22.2)
Amikacin	17 (60.7)	10 (50)	3 (33.3)

Table 4.

Relationship between biofilm-associated genes and biofilm production intensity.

Gene	High production	Intermediate production	Low production	*P*-value
	n (%)	n (%)	n (%)	
algD (n=49)	27 (55.1)	14 (28.6)	8 (16.3)	<0.05
pelA (n=39)	22 (56.4)	11 (28.2)	6 (15.4)	<0.05
pslA (n=34)	19 (55.9)	10 (29.4)	5 (14.7)	<0.05

Discussion

This study has made possible the identification of important new information regarding the resistance patterns, biofilm-forming capacities, and detection of virulent factors in *P. aeruginosa* isolates from burn infections. A significant percentage (63.1%) of the 57 isolates exhibited multidrug resistance (MDR), which is in line with worldwide trends of *P. aeruginosa* resistance in burn units according to Ugwuanyi et al.[13] The overuse and misuse of antimicrobials by patients leads to resistance to antibiotics.[14] Resistance to imipenem (87.7%) and ciprofloxacin (61.4%) was positively correlated with increased biofilm formation. These results align with those reported by Heidari and colleagues, who similarly noted high resistance to carbapenems and fluoroquinolones in biofilm-producing strains.[15] Additionally, isolates exhibiting elevated levels of *algD* and *pelA* expression demonstrated increased biofilm density, consistent with the findings of Ahmed et al,[16] who noted comparable correlations between gene expression and production of biofilm.The high rate of resistance found here is a serious challenge for empirical therapy. It requires adjustments to antibiotic regimens in clinical settings, as noted by Kumar et al.[17] The incidence of biofilm development was significant, with 84.1% of isolates exhibiting moderate or high biofilm formation capacity, confirming the findings of Yang et al.[18] Biofilms increase antibiotic resistance and immunological clearance, complicating the management of burn-associated infections.[19,20] The PCR gene expression analysis revealed that robust biofilm producers exhibited elevated levels of *algD*, *pelA*, and *pslA* compared to strains that were weak or did not form biofilms at all. Our investigation revealed that *algD* was elevated in 86.0% of isolates, aligning with the observations made by Häußler et al,[21] who highlighted its significance in alginate production and its persistence within host tissues. Similarly, *pelA* and *pslA*, which play a role in the synthesis of the polysaccharide matrix, were expressed in 68.4% and 59.6% of isolates, respectively. These findings are consistent with a study by Farhan et al.,[22] highlighting their collaborative function in biofilm structure. Antibiotic susceptibility testing revealed a significant decline in sensitivity to monotherapies, with resistance rates of ceftazidime (91.2%), imipenem (87.7%), gentamicin (73.7%), and ciprofloxacin (61.4%). The findings are consistent with a publication by de Sousa et al,[23] who found higher resistance rates for these drugs. The combination therapy, particularly the combination of ceftazidime-avibactam and colistin, had a significant synergistic impact, with an increase in inhibitory zones of more than 35% when compared to the individual medications. This observation is consistent with the synergistic combination effects reported by Mikhail et al.[24] A clear relationship was observed between the *algD*, *pelA*, and *pslA* genes and biofilm production and resistance patterns, matching the results reported by Rajabi et al.[25] In addition, a robust inverse relationship was observed between the mechanical stability of the biofilm and the susceptibility to single-agent antibiotic treatment, providing further evidence that the genetic control of biofilm architecture is a central mechanism driving drug resistance in this model, as recommended by de Sousa et al.[26] Our results highlight the significance of including molecular diagnostics in standard microbiological surveillance in burn units. Customized treatment regimens can be guided by identifying high-risk MDR *P. aeruginosa* strains with a high capacity to produce biofilms, as recommended by Martinez et al.[27] Our findings support the potential use of biofilm-targeted adjuvant medications and optimized combination regimens to enhance treatment outcomes. The robustness of our conclusions is further supported by the concordance of our results with international literature, even with a moderate sample size. To further understand the molecular mechanisms behind resistance and biofilm formation, future research should concentrate on longitudinal monitoring and the insertion of more virulent genes.

Conclusion

Our data support the substantial relationship between biofilm production, antibiotic resistance, and genes related to biofilm production in *P. aeruginosa* isolates from burn sites. Implementing gene-targeted techniques and optimal combination treatments may significantly enhance the control of infections and clinical outcomes in burn care facilities.

Ethical Approval

This study was approved by the Department of Applied Biological Science Committee/College of Biotechnology, Al-Nahrain University, under the approval number: 3, 2025.

Competing Interests

The authors of this article confirm that they have no conflicting interests.

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***Corresponding author**: Dr. Hasan A. Aal Owaif. Department of Applied Biological Science, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq. E-mail: hasan.abdulhadi@nahrainuniv.edu.iq*