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Research Paper

Impact of biofilm-forming ability and multidrug resistance on clinical outcomes of burn wound infections caused by *Pseudomonas aeruginosa*

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ABSTRACT

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Burn wound infections caused by *Pseudomonas aeruginosa* represent a major clinical challenge due to the pathogen's virulence, biofilm-forming capacity, and increasing multidrug resistance (MDR). These factors may adversely affect treatment efficacy and patient outcomes in burn units; however, their combined clinical impact remains insufficiently characterized in hospital-based settings. This study aimed to evaluate the association between biofilm-forming ability, MDR, and clinical outcomes in burn wound infections caused by *P. aeruginosa*. In this cross-sectional study, 30 non-duplicate *P. aeruginosa* isolates were obtained from hospitalized burn patients at a tertiary care burn center between March 2022 and March 2023. Bacterial identification was performed using standard microbiological methods. MDR was defined as resistance to at least one agent in three or more antimicrobial classes. Biofilm formation was assessed using the microtiter plate assay. Among the 30 patients, 76.7% were male, and the mean age was 45.8 ± 16.9 years. The mean total body surface area (TBSA) was 42.9% ± 20%. Fifteen isolates (50%) were classified as MDR. Biofilm assessment revealed that 40% of isolates were strong biofilm producers, 16.7% moderate, and 43.3% weak producers. No statistically significant associations were identified between biofilm strength, MDR status, TBSA, and clinical outcomes. *P. aeruginosa* burn wound infections were characterized by a high prevalence of biofilm formation and MDR. While no significant associations with mortality were demonstrated, trends toward poorer outcomes in patients infected with strong biofilm-producing and MDR isolates underscore the clinical relevance of these traits. Larger studies incorporating molecular analyses are warranted to clarify their prognostic significance.

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1. Introduction

Burn wound infections remain a major cause of morbidity and mortality among hospitalized patients, particularly in intensive care and burn units.

Disruption of the skin barrier, prolonged hospitalization, invasive procedures, and extensive antimicrobial exposure collectively predispose burn patients to severe bacterial infections and poor clinical outcomes [1]. Among the pathogens implicated, *Pseudomonas aeruginosa* is recognized as one of the most frequent and clinically challenging etiologic agents of burn wound infections due to its intrinsic resistance mechanisms and remarkable adaptive capacity [2].

P. aeruginosa possesses a broad collection of virulence determinants, including quorum sensing systems, secreted toxins, and biofilm-forming ability, which facilitate persistent colonization of burn wounds and impair host immune clearance [2, 3]. Biofilms are structured microbial communities embedded in an extracellular polymeric matrix that confer protection against environmental stressors, host defenses, and antimicrobial agents.

In the context of burn wounds, biofilm formation has been associated with delayed wound healing, increased risk of systemic infection, and prolonged hospital stay [4, 5].

In parallel, the emergence and dissemination of multidrug-resistant (MDR) *P. aeruginosa* have become a critical global health concern [6]. MDR strains, commonly defined as isolates resistant to at least one agent in three or more antimicrobial classes, significantly limit therapeutic options and are associated with increased treatment failure and mortality [7]. Burn units, characterized by high antibiotic pressure and vulnerable patient populations, represent an important reservoir for MDR *P. aeruginosa* [8].

Accumulating evidence suggests a complex and clinically relevant interplay between biofilm formation and antimicrobial resistance in *P. aeruginosa*. Biofilm-associated cells exhibit phenotypic tolerance to antibiotics, while MDR strains frequently demonstrate enhanced biofilm-forming capacity, further complicating infection management [9, 10]. However, the extent to which biofilm-forming ability and multidrug resistance jointly influence clinical outcomes in burn wound infections remains incompletely understood, particularly in hospital-based settings within low- and middle-income countries.

Understanding the relationship between biofilm production, multidrug resistance, and patient outcomes is essential for improving infection control strategies, guiding antimicrobial stewardship, and optimizing therapeutic interventions in burn care.

Therefore, this study aimed to evaluate the impact of biofilm-forming ability and multidrug resistance on clinical outcomes among burn wound infections caused by *P. aeruginosa* in hospitalized patients.

2. Materials and Methods

2.1 *Study Design and Setting*

This cross-sectional experimental study was conducted at the burn unit of Velayat Hospital, a tertiary referral center in northern Iran, and included patients hospitalized between March 2022 and March 2023. Medical records of burn patients with culture-confirmed *P. aeruginosa* burn wound infections were reviewed. A total of 30 non-duplicate clinical isolates obtained from 30 hospitalized burn patients were included in the analysis. Only the first isolate per patient was considered to avoid duplication. Demographic and clinical data were extracted from hospital records, including age, sex, total body surface area (TBSA) burned, length of hospital stay, and in-hospital mortality.

2.2 *Bacterial isolation and identification*

Burn wound samples were collected as part of routine clinical care using sterile swabs or tissue specimens. Samples were cultured on blood agar and MacConkey agar plates and incubated aerobically at 37 °C for 18–24 hours. Presumptive identification of *P. aeruginosa* was based on colony morphology, pigment production, oxidase positivity, and growth at 42 °C. Final identification was confirmed using standard biochemical tests, in accordance with standard microbiological protocols.

2.3 *Antimicrobial susceptibility testing*

Antimicrobial susceptibility testing was performed using the disk diffusion method on Mueller–Hinton agar, and results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. The antibiotics tested represented at least five antimicrobial classes commonly used against *P. aeruginosa*, including antipseudomonal penicillins, cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides. MDR was defined according to the international criteria proposed by Magiorakos et al., as resistance to at least one agent in three or more antimicrobial categories [12].

2.4 *Assessment of biofilm formation*

Biofilm-forming ability of *P. aeruginosa* isolates was evaluated using the microtiter plate assay, as previously described with minor modifications [13]. Briefly, isolates were grown overnight in tryptic soy broth (TSB) at 37 °C. The bacterial suspension was adjusted to 0.5 McFarland standard and diluted 1:100 in fresh TSB. Aliquots (100 µL) were inoculated into sterile flat-bottom 96-well polystyrene microtiter plates and incubated at 37 °C for 24 hours. Following incubation, wells were gently washed three times with phosphate-buffered saline (PBS) to remove planktonic cells and air-dried. Adherent biofilms were fixed with methanol, stained with 0.1% crystal violet for 10-15 minutes, and excess stain was removed by washing with distilled water. The bound dye was solubilized using 30% acetic acid, and optical density (OD) was measured at 550 nm using a microplate reader. Isolates were categorized as non-biofilm producers, weak, moderate, or strong biofilm producers based on OD values relative to the negative control, according to established criteria.

2.5 *Statistical analysis*

Statistical analyses were performed using SPSS software ver. 21 (IBM Corp, Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as mean ± standard deviation or median (interquartile range), as appropriate. Associations between biofilm-forming ability, MDR status, and clinical outcomes were assessed using the chi-square or Fisher's exact test for categorical variables. A P value < 0.05 was considered statistically significant.

3. Results

In this study, 30 *P. aeruginosa* isolates were tested, including 23 samples from male (76.7%) and 7 female (23.3%) patients. The mean age of the patients was 45.8 years (±16.9), with a range from 16 to 81 years. The average length of hospital stay was 13.1 days (±9.8), with a range of 2 to 36 days. The total body surface area (TBSA) affected by burns had an average of 42.9% (±20%), ranging from 13% to 83%. Of the isolates, 15 (50%) were classified as MDR, while the remaining 15 were non-MDR. The clinical outcomes of the patients were as follows: 11 (36.7%) patients died, and 19 (63.3%) were discharged. When considering biofilm production, 13 isolates (43.3%) were categorized as weak biofilm producers, 5 isolates (16.7%) exhibited moderate biofilm formation, and 12 isolates (40%) demonstrated strong biofilm production.

The correlation between biofilm production strength and patient outcome revealed that among the weak biofilm producers, 4 patients (30.8%) died and 9 (69.2%) were discharged. For those with strong biofilm production, 7 patients (41.2%) died and 10 (58.8%) were discharged. The difference in clinical outcomes between weak and strong biofilm-producing isolates was not statistically significant ($p = 0.71$). Regarding TBSA, there was no significant association between the extent of burn injury and the clinical outcome ($p = 0.47$). The impact of MDR on patient outcome was also assessed. Again, this difference was not statistically significant ($p = 0.46$). Table 1 illustrated the full results of associations between biofilm strength, TBSA, MDR status, and clinical outcomes.

4. Discussion

Clinical outcomes of burn wound infections are influenced by a complex interaction between host factors, bacterial virulence traits, and antimicrobial resistance patterns. Evaluating these factors in real-world hospital settings is essential for contextualizing laboratory findings and improving patient management strategies. The present study provides clinical insight into the role of biofilm formation in *P. aeruginosa* burn wound infections. The mortality rate observed in this cohort reflects the well-documented severity of *P. aeruginosa* infections in burn patients and is comparable to rates reported in similar hospital-based studies [14, 15].

Extensive tissue damage, immune dysregulation, and prolonged hospitalization create favorable conditions for persistent infections and systemic complications. Biofilm formation is a central virulence mechanism of *P. aeruginosa*, enabling long-term persistence in burn wounds and protection against host immune responses and antimicrobial agents [16]. In the present study, a substantial proportion of isolates demonstrated strong biofilm-forming capacity, while no statistically significant association was found between biofilm strength and mortality. Similar trends have been reported previously, suggesting that biofilm formation may contribute to clinical severity even when statistical significance is not achieved in small cohorts [17, 18].

MDR remains a critical challenge in burn units worldwide. Half of the isolates in this study were classified as MDR, consistent with reports from tertiary burn centers in comparable healthcare settings [19]. High antibiotic pressure and frequent use of broad-spectrum antimicrobials are recognized drivers of MDR *P. aeruginosa* emergence in burn units [20]. Although MDR status was not significantly associated with mortality in this study, previous studies have shown that MDR infections are associated with delayed appropriate therapy, prolonged hospitalization, and increased healthcare costs [21, 22]. The interaction between biofilm formation and antimicrobial resistance further complicates the management of *P. aeruginosa* infections.

Table 1. Associations between biofilm strength, TBSA, MDR status, and clinical outcomes

Variable	Group	Biofilm production weak No. (%)	Biofilm production strong No. (%)	P value
Outcome	Died	4 (30.8)	7 (41.2)	0.71
	Discharged	9 (69.2)	10 (58.8)	
TBSA	≤40%*	9 (69.2)	9 (52.9)	0.47
	>40%	4 (30.8)	8 (47.1)	
MDR	Yes	5 (38.5)	10 (58.8)	0.46
	No	8 (61.5)	7 (41.2)	

*Categorized based on the estimated median

Biofilm-associated bacteria exhibit phenotypic tolerance to antibiotics independent of classical resistance mechanisms, while MDR strains often demonstrate enhanced biofilm-forming capacity [16, 23]. The coexistence of these traits may limit treatment effectiveness and contribute to persistent infections, underscoring the need for integrated diagnostic and therapeutic approaches in burn care.

This study has several limitations that should be acknowledged. The retrospective design limits causal inference between bacterial characteristics and clinical outcomes. The relatively small sample size may have reduced the statistical power to detect significant associations. In addition, molecular characterization of resistance determinants and biofilm-associated genes was not performed.

In conclusion, *P. aeruginosa* as a major cause of burn wound infections, characterized by a high prevalence of biofilm formation and MDR. Although statistically significant associations with mortality were not observed, strong biofilm production and MDR phenotypes were more frequently associated with unfavorable clinical outcomes. These findings highlight the importance of continuous surveillance, targeted antimicrobial stewardship, and consideration of biofilm-related factors in the management of burn wound infections.

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Authors' contributions

Conceptualization, and supervision: HS, MM, HAB, MH. Data curation: MM, AO, NZ, MH. Investigation: AO, NZ. Methodology: HS, MH. Original draft preparation: HS, AO, NZ, MH. Critical revision and editing: HS, MM, HAB, MH. All authors read and approved the final version of manuscript.

Conflict of interest

No potential conflict of interest was reported by the authors.

Ethical declarations

This study design was approved by the Ethics Committee of Guilan University of Medical Sciences (Approval Code: IR.GUMS.REC.1401.411). Patient data were analyzed anonymously. The need for written informed consent was waived by the committee due to the design of the study.

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References

[1] Alharthi AF, Al Sulaiman K, Alotaibi S, Alqahtani R, Damfu N, Alharbi A, et al. Managing Infections in Burn Patients: Strategies and Considerations for Antimicrobial Dosing. Eur Burn J. 2025;6(4):53. DOI: 10.3390/ebj6040053 PMID: 41133485

[2] Hasannejad-Bibalan M, Jafari A, Sabati H, Goswami R, Jafaryparvar Z, Sedaghat F, et al. Risk of type III secretion systems in burn patients with Pseudomonas aeruginosa wound infection: A systematic review and meta-analysis. Burns. 2021;47(3):538-544. DOI: 10.1016/j.burns.2020.04.024 PMID: 32532479

[3] Miranda SW, Asfahl KL, Dandekar AA, Greenberg EP. Pseudomonas aeruginosa Quorum Sensing. Adv Exp Med Biol. 2022;1386:95-115. DOI: 10.1007/978-3-031-08491-1_4 PMID: 36258070

[4] Karygianni L, Ren Z, Koo H, Thurnheer T. Biofilm Matrixome: Extracellular Components in Structured Microbial Communities. Trends Microbiol. 2020;28(8):668-681. DOI: 10.1016/j.tim.2020.03.016 PMID: 32663461

[5] Maslova E, Eisaiankhongi L, Sjöberg F, McCarthy RR. Burns and biofilms: priority pathogens and in vivo models. NPJ Biofilms Microbiomes. 2021;7(1):73. DOI: 10.1038/s41522-021-00243-2 PMID: 34504100

[6] Dabbousi AA, Dabboussi F, Hamze M, Osman M, Kassem II. The Emergence and Dissemination of Multidrug Resistant Pseudomonas aeruginosa in Lebanon: Current Status and Challenges during the Economic Crisis. Antibiotics (Basel). 2022;11(5):687. DOI: 10.3390/antibiotics11050687 PMID: 35625331

[7] Cosentino F, Viale P, Giannella M. MDR/XDR/PDR or DTR? Which definition best fits the resistance profile of Pseudomonas aeruginosa? Curr Opin Infect Dis. 2023;36(6):564-571. DOI: 10.1097/QCO.0000000000000966 PMID: 37930070

[8] Aguilera-Sáez J, Andreu-Solà V, Larrosa Escartín N, Rodríguez Garrido V, Armadans Gil L, et al. Extensively drug-resistant Pseudomonas Aeruginosa outbreak in a burn unit: management and solutions. Ann Burns Fire Disasters. 2019;32(1):47-55. PMID: 31285735

[9] Cho HH, Kwon KC, Kim S, Park Y, Koo SH. Association between Biofilm Formation and Antimicrobial Resistance in Carbapenem-Resistant Pseudomonas Aeruginosa. Ann Clin Lab Sci. 2018;48(3):363-368. PMID: 29970441

[10] Silva A, Silva V, López M, Rojo-Bezares B, Carvalho JA, et al. Antimicrobial Resistance, Genetic Lineages, and Biofilm Formation in Pseudomonas aeruginosa Isolated from Human Infections: An Emerging One Health Concern. Antibiotics (Basel). 2023;12(8):1248. DOI: 10.3390/antibiotics12081248 PMID: 37627668

[11] CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 35th ed. CLSI Supplement M100. 2025. URL: https://clsi.org/shop/standards/m100-ed35/

[12] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81. DOI: 10.1111/j.1469-0691.2011.03570.x PMID: 21793988

[13] O'Toole GA. Microtiter dish biofilm formation assay. J Vis Exp. 2011;(47):2437. DOI: 10.3791/2437 PMID: 21307833

[14] Biswal I, Arora BS, Kasana D, Neetushree. Incidence of multidrug resistant pseudomonas aeruginosa isolated from burn patients and environment of teaching institution. J Clin Diagn Res. 2014;8(5):DC26-9. DOI: 10.7860/JCDR/2014/7483.4383 PMID: 24995179

[15] Heydari AA, Jamialahmadi S, Kouhi Noghondar M. Three-Year Evaluation of Pseudomonas aeruginosa Bacteremia in Patients Admitted to a University-Affiliated Hospital, Mashhad, Iran. Jundishapur J Microbiol. 2022;15(7):e126998.
DOI: 10.5812/jjm-126998

[16] Singh P, Arsi L. Pseudomonas aeruginosa Biofilms: Pathogenicity, Clinical Challenges and Emerging Therapeutic Strategies. The Microbe. 2025;100645.
DOI: 10.1016/j.microb.2025.100645

[17] Wang Y, Xiao Q, Yang Q, Long Y, Jiang Z, et al. Impact of Pseudomonas aeruginosa biofilm formation by different sequence types on treating lower limb vascular infections. Curr Res Microb Sci. 2025;8:100379.
DOI: 10.1016/j.crmicr.2025.100379 PMID: 40395473

[18] Jordana-Lluch E, Escobar-Salom M, Torrens G, Barceló IM, Estévez MÁ, et al. Corticosteroids modulate biofilm formation and virulence of Pseudomonas aeruginosa. Biofilm. 2025;9:100289.
DOI: 10.1016/j.bioflm.2025.100289 PMID: 40539031

[19] Tchakal-Mesbahi A, Abdouni MA, Metref M. Prevalence of Multidrug-Resistant Bacteria Isolated From Burn Wounds In Algeria. Ann Burns Fire Disasters. 2021;34(2):150-156.
PMID: 34584503

[20] Elfadadny A, Ragab RF, AlHarbi M, Badshah F, Ibáñez-Arancibia E, et al. Antimicrobial resistance of Pseudomonas aeruginosa: navigating clinical impacts, current resistance trends, and innovations in breaking therapies. Front Microbiol. 2024;15:1374466.
DOI: 10.3389/fmicb.2024.1374466 PMID: 38646632

[21] Kalın G, Alp E, Chouaikhi A, Roger C. Antimicrobial Multidrug Resistance: Clinical Implications for Infection Management in Critically Ill Patients. Microorganisms. 2023;11(10):2575.
DOI: 10.3390/microorganisms11102575 PMID: 37894233

[22] Hirsch EB, Tam VH. Impact of multidrug-resistant Pseudomonas aeruginosa infection on patient outcomes. Expert Rev Pharmacoecon Outcomes Res. 2010;10(4):441-51.
DOI: 10.1586/erp.10.49 PMID: 20715920

[23] Dutt Y, Dhiman R, Singh T, Vibhuti A, Gupta A, et al. The Association between Biofilm Formation and Antimicrobial Resistance with Possible Ingenious Bio-Remedial Approaches. Antibiotics (Basel). 2022;11(7):930.
DOI: 10.3390/antibiotics11070930 PMID: 35884186