

Phenotypic biofilm formation and detection of biofilm-associated genes in multidrug-resistant *Pseudomonas aeruginosa* isolated from burn wounds

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Abstract: Background: *Pseudomonas aeruginosa* is widely recognized as a ubiquitous, drug-resistant, biofilm-forming bacterium that produces numerous virulence factors and plays a prominent role in hospital-acquired infections. **Objectives:** This study aimed to determine the prevalence of multidrug resistance (MDR), biofilm formation and biofilm-associated genes among *P. aeruginosa* isolates recovered from burn wound infections. **Methods:** A prospective cross-sectional observational study was conducted in which total of 176 burn wound samples were collected and processed for bacterial isolation. Identification of isolates was confirmed by polymerase chain reaction (PCR) targeting the *oprL* gene. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Biofilm-forming ability was assessed using the microtiter plate assay. Furthermore, PCR was used to detect biofilm-associated genes, including *pelA*, *pelD*, *cupA*, *algD*, *pelF*, *pslA*, *pslB* and *pslD*, in biofilm-producing isolates. **Results:** Out of 176 burn wound samples, 74 (42%) were positive for *P. aeruginosa*, among which 58 isolates were identified as MDR while all isolates remained susceptible to polymyxin B. Biofilm formation was observed in all MDR isolates, with 13.79%, 44.82% and 41.37% categorized as weak, moderate and strong biofilm producers, respectively, whereas only a limited proportion of non-MDR isolates demonstrated biofilm-forming ability ($P = 0.001$). Among MDR biofilm-forming isolates, biofilm-associated genes such as *pslA* and *algD* were each detected in 96.55% of isolates while *cupA* were identified in 94.82% of isolates. Notably, *cupA* showed a statistically significant association with biofilm-forming intensity ($P = 0.020$), suggesting its potential role in enhanced biofilm establishment among MDR isolates. **Conclusion:** Overall, the high prevalence of multidrug-resistant, biofilm-forming *P. aeruginosa* isolates recovered from burn wounds is an important microbiological concern. The frequent coexistence of multidrug resistance and biofilm-forming capacity observed in this study suggests a possible association that may complicate infection management and emphasizes the importance of continuous surveillance and effective infection control strategies.

Keywords: Burns; Biofilms; Multidrug resistant; *Pseudomonas aeruginosa*; Wound Infection

Submitted on 09-04-2026 – Revised on 19-05-2026 – Accepted on 30-06-2026

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacterium commonly found in diverse environmental and clinical niches, including soil, water, plants, sewage, animals, humans and medical devices (Letizia *et al.*, 2025). It is responsible for a wide range of community-acquired and hospital-acquired infections, including bacteremia, surgical site infections, pneumonia, ventilator-associated pneumonia, urinary tract infections, otitis media, burn wound infections and keratitis (Ito and Sugai, 2021). *P. aeruginosa* predominantly affects immunocompromised individuals and possesses multiple virulence mechanisms that facilitate adhesion, colonization, immune evasion and biofilm formation. Patients with severe burns, cystic fibrosis and cancer are particularly vulnerable to infections caused by this pathogen, which are often associated with increased morbidity and mortality (Turkina and Vickstrom, 2019). The emergence of

multidrug-resistant (MDR) *P. aeruginosa* strains further complicates therapeutic management and represents a significant challenge in clinical settings (Dogonchi *et al.*, 2018).

Bacteria colonize biotic and abiotic surfaces by forming biofilms, initially as microcolonies encased in extracellular polymeric substances, which serve as protective matrices against environmental threats. Within this biofilm matrix, cells demonstrate enhanced resistance to host immune responses, including phagocytosis and increased tolerance to oxidative stress, nutrient and oxygen limitations, accumulation of metabolic byproducts, interspecies competition and conventional antimicrobial therapies (Almatroudi, 2025). Biofilm-associated infections are a significant clinical concern and have been reported to contribute substantially to hospital-acquired and chronic microbial infections in humans. The ability of *Pseudomonas aeruginosa* to form robust biofilms is recognized as an important factor associated

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with its persistence and antimicrobial tolerance in clinical settings (Römling and Balsalobre, 2012).

Biofilm formation is considered an important virulence factor of *Pseudomonas aeruginosa* that contributes to its persistence and pathogenicity in clinical settings by enhancing tolerance to antibiotics, disinfectants and adverse environmental conditions (Garousi *et al.*, 2022). Within biofilms, bacterial cells are embedded in a self-produced extracellular polymeric matrix composed of extracellular DNA, proteins and exopolysaccharides. Among the major exopolysaccharides in the *P. aeruginosa* biofilm matrix, alginate, Pel and Psl are recognized as key structural components (Roy *et al.*, 2013). Alginate contributes to the structural stability and protective properties of biofilms by forming a hydrated polymeric matrix that protects bacterial cells from environmental stress and host immune responses. The genes involved in alginate biosynthesis are organized within a large operon, with *algD* as an important gene involved in the initiation of alginate production (Gheorghita *et al.*, 2023). Similarly, *Psl* plays an essential role in initial biofilm development and structural stability, whereas *Pel*, a glucose-rich polysaccharide, contributes to biofilm architecture and density (Wei and Ma, 2013).

Furthermore, the *cup* gene system encodes chaperone-usher pathway fimbriae involved in bacterial adhesion to surfaces and the early stages of biofilm formation (Thi *et al.*, 2020). Consequently, biofilm-associated infections caused by *P. aeruginosa* are often difficult to eradicate and may contribute to reduced antimicrobial effectiveness and persistent infections (Tuon *et al.*, 2022, Li *et al.*, 2023). Based on these well-established roles in biofilm formation and structural integrity, key genes involved in these pathways (*pel*, *psl*, *alg* and *cup* operons) were selected for molecular detection in the present study to assess their distribution among multidrug-resistant clinical isolates (Thi *et al.*, 2020).

Although several studies have reported the prevalence of multidrug-resistant *Pseudomonas aeruginosa* in burn wound infections, limited information is available regarding the combined phenotypic and molecular characterization of biofilm formation among MDR isolates in Pakistan. Therefore, the present study was designed to explore the prevalence of biofilm formation and the distribution of selected biofilm-associated genes, particularly those involved in exopolysaccharide production and adhesion (*pel*, *psl*, *alg* and *cup* operons), among MDR *P. aeruginosa* to evaluate their possible association with biofilm-forming capacity. The findings of this study may contribute to a better understanding of the molecular characteristics associated with biofilm formation and support future infection control and antimicrobial management strategies.

MATERIALS AND METHODS

Study design and settings

This was a prospective cross-sectional observational study conducted at major burn centers in Punjab, Pakistan, including the Burn & Reconstructive Surgery Center, Allied Hospital, Faisalabad and the Modern Burn Unit, Nishtar Medical University, Multan, from July 2023 to June 2024. The study included patients of all age groups with varying degrees of burn injuries, including thermal, chemical and electrical burns. A non-probability convenience sampling method was employed for sample collection. Inclusion criteria comprised patients with confirmed burn wounds and no prior long-term antibiotic treatment before admission. Only non-duplicate clinical isolates of *Pseudomonas aeruginosa* obtained from burn wound samples were included in the study. Patients with incomplete clinical records or those who refused consent were excluded. Isolates showing contamination or incomplete identification were also excluded from the analysis. To minimize potential selection bias, standardized inclusion and exclusion criteria and uniform laboratory identification procedures were applied consistently to all collected samples throughout the study period.

The sample size was estimated using the standard formula for prevalence studies, as described by Thrusfield (2007), with a 95% confidence level, an expected prevalence (*P*_{exp}) of 27% based on previous studies and a precision level (*d*) of 7%. The minimum calculated sample size was 154; therefore, a total of 176 burn wound samples were included in the study.

$$n = 1.96^2 P_{exp} (1 - P_{exp}) / d^2$$

Where *n* = required sample size, *P*_{exp} = expected prevalence and *d* = desired precision.

A total of (*n*=176) burn wound samples were collected from patients by trained medical personnel using sterile cotton swabs pre-moistened with peptone water. Informed consent was obtained from the patients or their guardians after explaining the study objectives. The swabs were immediately transported to the microbiology laboratory under aseptic conditions and subsequently streaked onto *Pseudomonas* Cetrimide agar plates and incubated at 37°C overnight (Abdulhaq *et al.*, 2020). Colonies showing characteristic morphology of *Pseudomonas aeruginosa* such as greenish pigmentation, fruity odor and growth on cetrimide agar were subjected to further identification using standard biochemical tests including oxidase activity, catalase activity and motility. The confirmed isolates were stored in sterile peptone water containing 20% glycerol at -80°C for further analysis (Abbas *et al.*, 2022).

Detection and identification of *Pseudomonas aeruginosa*

The phenotypically confirmed *P. aeruginosa* isolates were cultured in Luria Broth overnight at 37°C prior to DNA extraction. Genomic DNA was extracted using a commercial kit (Thermo Scientific, USA). Following that the extracted DNA samples were kept at -20 °C. Polymerase chain reaction (PCR) was then applied for molecular confirmation of *P. aeruginosa* isolates using *oprL* gene primers (Table 1). Briefly, a 25 µL reaction mixture was prepared, including 12.5 µL of master mix, 1 µL each forward and reverse primer, 4 µL template DNA and 6.5 µL nuclease-free water. The PCR thermal profile was adjusted to 94 °C (2 min), 94 °C (30 sec), 62 °C (30 sec), 72 °C (30 sec) and 72 °C (10 min) × 35 cycles, as described by Okafor and Nwodo (2023). The *P. aeruginosa* reference strain ATCC 27853 was used as the positive control and distilled water as the negative control. The amplified PCR products were separated on 1.2% agarose gel stained with ethidium bromide and visualized using a gel documentation system (Thermo Scientific, UK).

Detection of antibiotic resistance among *Pseudomonas aeruginosa*

The antimicrobial susceptibility testing was carried out using the Kirby–Bauer disk diffusion method targeting 9 antimicrobial agents representing different antibiotic classes including: imipenem (10 µg), meropenem (10 µg), amoxicillin–clavulanic acid (20/10 µg), amikacin (30 µg), gentamicin (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg) and aztreonam (30 µg) as described by (Bauer et al., 1966). A 0.5 McFarland bacterial suspension of *P. aeruginosa* was used for inoculation. The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of polymyxin B against the *P. aeruginosa* isolates (Madni et al., 2025). The results were interpreted according to CLSI guidelines (CLSI, 2023). Multidrug resistance was defined as resistance to at least one agent in three or more antimicrobial categories according to standard international criteria.

Phenotypic detection of biofilm production by *Pseudomonas aeruginosa*

The biofilm formation among *P. aeruginosa* isolates was evaluated using the microtiter plate assay (MPA). The *P. aeruginosa* ATCC 27853 and sterile tryptic soy broth were served as positive and negative controls, respectively. Biofilm formation was quantified by measuring the optical density (OD) at 594 nm using a microplate reader (Thermo Scientific). All samples, including blank and controls, were run in triplicate. A cut-off value (ODc) was determined as ODc = average OD of negative control + (3 × negative control SD). The isolates were categorized into 4 different OD-based categories: nonbiofilm producer (OD < ODc); weak biofilm producer

(ODc < OD < 2 × ODc); moderate biofilm producer (2 × ODc < OD < 4 × ODc); strong biofilm producer (4 × ODc < OD) as described by (Banar et al., 2016).

Detection of biofilm associated genes in *Pseudomonas aeruginosa*

The distribution of biofilm-associated genes was investigated among the biofilm-producing *P. aeruginosa* isolates. The genomic DNA obtained from these isolates was used as a template for polymerase chain reaction (PCR) amplification of selected biofilm-associated genes, including *pelA*, *pelD*, *pelF*, *pslA*, *pslB*, *pslD*, *algD* and *cupA*. Briefly, a 25 µL reaction mixture was prepared, including 12.5 µL of master mix, 1 µL each forward and reverse primer, 4 µL template DNA and 6.5 µL nuclease-free water. The PCR thermal profile was adjusted to 94 °C (2 min), 94 °C (30 sec), 52-63 °C (30 sec) depending on the target gene, 72 °C (30 sec) and 72 °C (10 min) × 35 cycles. The amplified PCR products were separated on a 1.2% agarose gel stained with ethidium bromide and visualized using a gel documentation system (Thermo Scientific, UK). The primer sequences used for amplification of these genes are presented in (Table 1).

Statistical analysis

All collected data were organized in a Microsoft Excel spreadsheet and subsequently analyzed using Stata version 12 (StataCorp, USA). Statistical analysis was performed using the Chi-square test/Fisher's exact test to determine the association between variables. A p-value of less than 0.05 was considered statistically significant (Naz et al., 2018).

RESULTS

The current study included (n=176) burn wound samples among which 74 were confirmed as *P. aeruginosa* resulting in 42% prevalence. According to the CLSI criteria, the isolates showed high levels of resistance to several antibiotics, including ceftriaxone (94.60%), imipenem (91.90%), meropenem (89.18%) and aztreonam (81.08%) while all the isolates showed sensitivity to polymyxin B (100%) on the basis of broth micro dilution method as there MIC value was detected ≤ 4 µg/mL, as shown in (Table 2). The overall prevalence of multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) was 78.37% (58/74), while 21.62% (16/74) of the isolates were non-MDR.

The biofilm-forming ability of *P. aeruginosa* isolates was evaluated, showing that (67/74) of the isolates produced biofilms to varying degrees. Among these, 12.16% were categorized as weak, 44.59% as moderate and 33.78% as strong biofilm producers, while 9.45% were unable to produce biofilms. Among the MDR-PA (n=58) isolates, all strains exhibited biofilm production, with 13.79%, 44.82% and 41.37% classified as weak, moderate and strong biofilm producers, respectively.

Table 1: Sequences and product size of oligonucleotide primers used for PCR test.

Primer names	Primer sequences	Product size	Reference
<i>oprL</i> -F	ATGGAAATGCTGAAATTCGGC	504bp	(Okafor and Nwodo, 2023)
<i>oprL</i> -R	CTTCTTCAGCTCGACGCGACG		
<i>PelA</i> -F	CCTTCAGCCATCCGTTCTTCT	118bp	(Kabir <i>et al.</i> , 2024)
<i>PelA</i> -R	TCGCGTACGAAGTCGACCTT		
<i>PelD</i> -F	CTCATGAAACGCACCCCTCT	295bp	(Kabir <i>et al.</i> , 2024)
<i>PelD</i> -R	TGCGACCGATGAACGGATAG		
<i>PelF</i> -F	GAGGTCAGCTACATCCGTCG	789bp	(Banar <i>et al.</i> , 2016)
<i>PelF</i> -R	TCATGCAATCTCCGTGGCTT		
<i>PslA</i> -F	TGGGTCTTCAAGTTCCGCTC	119bp	(Kabir <i>et al.</i> , 2024)
<i>PslA</i> -R	ATGCTGGTCTTGCGGATGAA		
<i>PslB</i> -F	GCTTCAAGATCAAGCGCATC	160bp	(Masoumi and Keshavarzi, 2024)
<i>PslB</i> -R	ACCTCGATCATCACCAGGTC		
<i>PslD</i> -F	AAGTCAGCGGCAACAACAC	208bp	(Banar <i>et al.</i> , 2016)
<i>PslD</i> -R	CGCTTCTCCCAGTACCTCAA		
<i>AlgD</i> -F	CTACATCGAGACCGTCTGCC	593bp	(Murugan <i>et al.</i> , 2016)
<i>AlgD</i> -R	GCATCAACGAACCGAGCATC		
<i>CupA</i> -F	AATTTCGATGATCGCCTGTT	345bp	(Murugan <i>et al.</i> , 2016)
<i>CupA</i> -R	GCGATAGAGGTTGGTGTCGT		

Table 2: Percentage antibiotic resistance pattern of *Pseudomonas aeruginosa* isolates from burn wounds.

Antibiotics	Susceptibility pattern		
	Sensitive	Intermediate	Resistant
Amikacin	30 (40.54%)	14 (18.91%)	30 (40.54%)
Gentamicin	16 (21.62%)	10 (13.51%)	48 (64.86%)
Ceftriaxone	02 (2.70%)	02 (2.70%)	70 (94.60%)
Ceftazidime	16 (21.62%)	14 (18.91%)	44 (59.45%)
Imipenem	02 (2.70%)	04 (5.40%)	68 (91.90%)
Meropenem	02 (2.70%)	06 (8.10%)	66 (89.18%)
Amoxicillin/clavulanic acid	12 (16.21%)	18 (24.32%)	44 (59.45%)
Ciprofloxacin	24 (32.43%)	10 (13.51%)	40 (54.05%)
Aztreonam	04 (5.40%)	10 (13.51%)	60 (81.08%)
Polymyxin B	74 (100%)	00 (00.00%)	00 (00.00%)

Table 3: Distribution of biofilm formation among MDR and non-MDR *Pseudomonas aeruginosa* isolates.

Biofilm formation	No of isolates	% of isolates	Biofilm formation among MDR isolates	% of isolates	Biofilm formation among Non-MDR isolates	% of isolates
None	07	09.45%	00	00.00%	07	43.75%
Weak	09	12.16%	08	13.79%	01	06.25%
Moderate	33	44.59%	26	44.82%	07	43.75%
Strong	25	33.78%	24	41.37%	01	06.25%
Total	74	100%	58	100%	09	100%
Chi-square/ Fisher's exact test	$\chi^2 = 18.62$ (P < 0.001)					

Statistically significant at P < 0.05

In contrast, among the (n=16) non-MDR isolates, nine demonstrated biofilm production, whereas seven did not produce biofilms. However, the association between multidrug resistance and biofilm formation was statistically significant (P < 0.05), as shown in (Table 3).

The distribution of biofilm-associated genes among multidrug-resistant *Pseudomonas aeruginosa* isolates

demonstrated that *pslA* and *algD* were the most frequently detected genes, each identified in 56/58 (96.55%) isolates, followed by *cupA* in 55/58 (94.82%) and *pelA* in 52/58 (89.65%) isolates. In contrast, *pslB* showed the lowest prevalence, being detected in 21/58 (36.20%) isolates. Analysis of biofilm-forming intensity revealed that several genes occurred at higher frequencies among moderate- and strong-biofilm-forming isolates.

Table 4: Association between biofilm-forming phenotypes and biofilm-associated genes among multidrug-resistant *Pseudomonas aeruginosa* isolates.

Biofilm forming MDR isolates	<i>pelA</i>	<i>pelD</i>	<i>pelF</i>	<i>pslA</i>	<i>pslB</i>	<i>pslD</i>	<i>algD</i>	<i>cupA</i>
Weak (n=08)	06	05	05	07	03	03	08	06
Moderate (n=26)	24	13	22	25	10	15	26	25
Strong (n=24)	22	10	20	24	08	12	22	24
Total (n=58)	52	28	47	56	21	30	56	55
	(89.65%)	(48.27%)	(81.03%)	(96.55%)	(36.20%)	(51.72%)	(96.55%)	(94.82%)
χ^2 value	3.35	1.09	2.08	2.83	0.14	1.04	2.93	7.81
P-value	0.186	0.577	0.352	0.241	0.928	0.592	0.230	0.020*

*Statistically significant at $P < 0.05$

Among the investigated biofilm-associated genes, only *cupA* showed a statistically significant association with biofilm-forming intensity ($P = 0.020$), whereas no significant associations were observed for the remaining genes as shown in (Table 4).

DISCUSSION

Pseudomonas aeruginosa is a major opportunistic pathogen associated with a wide range of hospital-acquired infections, particularly surgical site and burn wound infections. Disruption of the skin barrier following burn injuries compromises both cellular and humoral immune defenses, thereby facilitating microbial colonization and infection at the wound site. In the present study, 74 out of 176 burn wound samples (42%) were positive for *P. aeruginosa*. Comparable prevalence rates have been reported in previous regional studies, including (Abdelraheem et al., 2020) from Egypt, who documented a prevalence of 45%, as well as studies conducted in Pakistan by (Ahsan et al., 2026), (Abdulhaq et al., 2020) and (Ijaz et al., 2019), which stated the prevalence rates of 48%, 38% and 36%, respectively. Similar findings have also been reported in a recent Pakistani study by (Azam et al., 2024), highlighting the continued predominance of *P. aeruginosa* in burn wound infections. The high occurrence of *P. aeruginosa* in burn wound infections may be associated with its remarkable environmental adaptability, intrinsic resistance mechanisms, prolonged hospital stays and challenges related to infection control practices in healthcare settings.

The antimicrobial susceptibility profile of *Pseudomonas aeruginosa* isolates in the present study demonstrated high levels of resistance to several commonly used antibiotics, particularly carbapenems, including imipenem (91.89%) and meropenem (89.18%), as well as ceftriaxone (94.60%). These findings are consistent with previous reports from Pakistan by Qureshi et al. (2018), Ijaz et al. (2019) and Abdulhaq et al. (2020), which also documented high resistance rates among clinical *P. aeruginosa* isolates. Comparable resistance trends have additionally been reported in neighboring countries,

including Nepal (Kunwar et al., 2021), Iran (Ghasemian et al., 2023) and Iraq (Polse et al., 2023), indicating the increasing regional burden of multidrug-resistant *P. aeruginosa*. Recent studies from burn units in Iran and Bangladesh have likewise highlighted the emergence and dissemination of carbapenem-resistant and extensively drug-resistant *P. aeruginosa* strains among burn patients (Akrami et al., 2024, Mondol et al., 2024). The observed high resistance to β -lactams and carbapenems may be associated with the extensive empirical use of broad-spectrum antibiotics in hospital settings, particularly in burn care units. In contrast, all isolates in the present study remained susceptible to polymyxin B (100%), which is in agreement with findings reported by (Maharjan et al., 2022) in Nepal, (Shukla et al., 2022) in India and studies from Pakistan by (Ijaz et al., 2019) and (Qureshi et al., 2018).

A high prevalence of multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) was observed in the present study, 58/74 (78.37%) of isolates were classified as MDR. This prevalence is comparable to findings reported from Iraq by (Polse et al., 2023), who documented an MDR rate of 76.06%, as well as studies from Brazil (Silva et al., 2017) and Egypt (Kishk et al., 2020), which reported MDR frequencies of 71.4% and 70%, respectively. However, the prevalence observed in the current study was higher than that reported by Ijaz et al. (2019) in Pakistan (58.6%) and by Shukla et al. (2022) in India (52%). Similar high frequencies of MDR *P. aeruginosa* among burn wound isolates have also been highlighted in recent studies from South Asia and the Middle East, reflecting the growing therapeutic challenges posed by resistant strains in burn care settings (Maher et al., 2026, Faris et al., 2026). The increased occurrence of MDR-PA, particularly resistance to β -lactam and carbapenem antibiotics, may be associated with multiple contributing factors, including extensive empirical antibiotic use, self-medication practices, horizontal transfer of resistance determinants, bacterial genetic adaptations and challenges related to infection control measures in healthcare settings.

In the present study, biofilm formation was detected in 67/74 (90.54%) *Pseudomonas aeruginosa* isolates, while

all multidrug-resistant isolates demonstrated varying degrees of biofilm-forming capacity ranging from weak to strong producers. Similar high frequencies of biofilm-forming *P. aeruginosa* isolates have been reported in studies from Korea (Cho *et al.*, 2018), Cameroon (Chimi *et al.*, 2024) and Iran (Motevasel *et al.*, 2024). Comparable findings have also been reported by Kamali *et al.* (2020), El-Sayed and Fahmy (2021) and Rajabi *et al.* (2022), supporting the widespread occurrence of biofilm-forming *P. aeruginosa* in clinical settings. Variations in the degree of biofilm formation among isolates reported across different studies may be influenced by differences in sample size, clinical source of isolates, infection severity, environmental conditions and methodologies used for biofilm detection and interpretation.

Among multidrug-resistant, biofilm-forming *Pseudomonas aeruginosa* isolates, the most frequently detected biofilm-associated genes were *pslA* and *algD*, each identified in 56/58 (96.55%) isolates, followed by *cupA*, detected in 55/58 (94.82%) isolates. The high prevalence of *pslA* observed in the present study is consistent with previous reports by Wahyudi *et al.* (2019), Abdulhaq *et al.* (2020) and Motevasel *et al.* (2024), which also documented the frequent occurrence of this gene among biofilm-producing *P. aeruginosa* isolates. The *psl* operon has been reported to play an important role in bacterial attachment, microcolony formation and maintenance of biofilm architecture (Ma *et al.*, 2006; Ma *et al.*, 2009). Similarly, the high prevalence of the *algD* gene is consistent with findings reported by Banar *et al.* (2016), Namuq *et al.* (2019) and Kamali *et al.* (2020). The *algD* gene is associated with alginate biosynthesis, which contributes to the extracellular polymeric matrix and may enhance biofilm structural stability (Ghafoor *et al.*, 2011). Likewise, the high frequency of *cupA* observed in the present study is comparable to previous findings reported by Abdelraheem *et al.* (2020) and Motevasel *et al.* (2024). Previous studies have also highlighted the importance of *cupA*-associated fimbrial systems in bacterial adhesion and early biofilm establishment among clinical *Pseudomonas aeruginosa* isolates (Ruer *et al.*, 2007). The *cupA* gene has been associated with bacterial adhesion during the early stages of biofilm development (Vallet *et al.*, 2001). Furthermore, the statistical analysis in the present study demonstrated a significant association between *cupA* and biofilm-forming intensity ($P = 0.020$), whereas the remaining investigated genes did not show a statistically significant association.

The biofilm-associated gene *pelA* was also detected at a high frequency among the biofilm-forming *Pseudomonas aeruginosa* isolates, occurring in 52/58 (89.65%) isolates. Similar prevalence patterns have been reported in more recent studies by Rajabi *et al.* (2022), Ghasemian *et al.* (2023) and Motevasel *et al.* (2024), which also

documented frequent detection of *pelA* among clinical biofilm-producing isolates. The *pelA* gene is considered important for biofilm matrix formation and maintenance of biofilm architecture. Other biofilm-associated genes, including *pelD*, *pelF*, *pslB* and *pslD*, were detected at variable frequencies, consistent with distributions reported in earlier studies (Banar *et al.*, 2016; Rajabi *et al.*, 2022). Variations in gene prevalence across studies may be influenced by differences in geographical region, clinical source of isolates, sample size and methodological approaches. Although the presence of these genes among clinical isolates may indicate their involvement in biofilm formation, the current findings are limited to gene detection and do not confirm functional gene expression or direct mechanistic roles.

Given the increasing prevalence of multidrug-resistant, biofilm-forming *Pseudomonas aeruginosa* isolates, alternative therapeutic approaches targeting biofilm formation are attracting considerable attention. Several strategies, including anti-biofilm compounds, quorum-sensing inhibitors, antimicrobial peptides and nanoparticle-based drug delivery systems, have shown promising potential in disrupting biofilm architecture and enhancing antimicrobial efficacy. Such approaches may offer therapeutic options for the management of persistent burn wound infections caused by MDR *P. aeruginosa* (Yadav *et al.*, 2023; Singh *et al.*, 2024).

The present study has certain limitations that should be considered while interpreting the findings. First, the study primarily focused on the microbiological and molecular characterization of multidrug-resistant *Pseudomonas aeruginosa* isolates and detailed demographic and clinical metadata such as patient age, gender, burn severity, treatment outcomes and duration of hospitalization were not included. Therefore, direct clinical correlations could not be established. Secondly, the study evaluated the presence of biofilm-associated genes using conventional PCR, which detects gene carriage rather than gene expression or functional activity. Additionally, the convenience sampling may limit the generalizability of the findings because the study included only patients attending selected burn centers and the sample may not be representative of the broader target population. This bias could have influenced the observed frequencies of *P. aeruginosa*, multidrug resistance and biofilm formation in either direction; however, its magnitude could not be quantified because probability sampling was not used and no external representative population was available for comparison. Future studies incorporating larger sample sizes, detailed clinical parameters and gene expression analysis are recommended to understand better the clinical and molecular significance of biofilm-associated genes in burn wound infections.

CONCLUSION

This study highlights the high prevalence of multidrug-resistant *Pseudomonas aeruginosa* among burn wound isolates, with a substantial proportion demonstrating moderate to strong biofilm-forming ability. The widespread detection of biofilm-associated genes, particularly *pslA*, *algD*, *cupA* and *pelA*, indicates a high distribution of these genes among clinical isolates and suggests a possible association with biofilm-forming phenotypes. The coexistence of multidrug resistance and biofilm-forming capacity observed in the present study may contribute to bacterial persistence and therapeutic challenges in burn wound management. These findings emphasize the importance of continuous surveillance of antimicrobial resistance patterns and biofilm-associated genes in *P. aeruginosa* clinical isolates. However, as the present study was limited to PCR-based gene detection, further molecular studies investigating gene expression and functional activity are recommended to better understand the role of these genes in biofilm development and pathogenicity.

Acknowledgments

The authors sincerely acknowledge the staff of the participating burn centers for their assistance during sample collection, the laboratory personnel for their technical assistance and all patients who participated in this study.

Authors' contributions

MAZ and ZN (Conception & design), MS and SM (Data collection and Manuscript drafting) NA and RA (Analysis and Interpretation) ABS and ZN (Analysis and Revision)

Funding

The authors received no specific funding for this work.

Data availability statement

The underlying research material can be requested from the corresponding author on demand.

Ethical approval

The Institutional Ethics Review Committee of Government College University Faisalabad, Pakistan, approved this study (Approval Code: GCUF/ERC/23/03B) on December 1, 2023. All procedures were conducted following international ethical standards and safety regulations. This study was performed in adherence with the STROBE guidelines. See supplementary file for the STROBE checklist.

Conflict of interest

Authors declare there is no conflict of interest of any type with regard to this study.

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