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Original Research Article

ASSOCIATION BETWEEN BIOFILM FORMATION, VIRULENCE FACTORS, AND MULTIDRUG RESISTANCE AMONG CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA

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ABSTRACT

Background: Pseudomonas aeruginosa is an important opportunistic pathogen capable of producing biofilms and multiple virulence factors that contribute to persistent infections and multidrug resistance (MDR). Understanding the relationship between these characteristics is essential for effective clinical management. The aim is to evaluate the association between biofilm formation, virulence factors, and multidrug resistance among clinical isolates of Pseudomonas aeruginosa. **Materials and Methods:** A hospital-based cross-sectional analytical study was conducted on 160 non-duplicate clinical isolates of Pseudomonas aeruginosa. Isolates were identified using standard microbiological methods. Biofilm formation was assessed by the tissue culture plate method, while virulence factors including hemolysin, protease, phospholipase, pyocyanin, and DNase production were evaluated phenotypically. Antimicrobial susceptibility testing was performed according to CLSI guidelines, and multidrug resistance was determined. Statistical analysis was performed using SPSS version 26.0, with $p<0.05$ considered statistically significant. **Result:** Pus and wound swabs accounted for the highest proportion of isolates (33.8%). Biofilm formation was detected in 76.2% of isolates, with moderate biofilm producers being the most common. Protease (78.8%), hemolysin (73.8%), and pyocyanin (70.0%) were the predominant virulence factors. Multidrug resistance was observed in 45.0% of isolates. Strong biofilm producers demonstrated the highest prevalence of MDR, and a statistically significant association was found between biofilm formation and multidrug resistance ($p<0.001$). **Conclusion:** Biofilm formation is strongly associated with multidrug resistance in Pseudomonas aeruginosa. The coexistence of multiple virulence factors and MDR highlights the pathogenic potential of these isolates. Routine evaluation of biofilm production and virulence characteristics may improve therapeutic decision-making, enhance infection-control practices, and help reduce the burden of resistant P. aeruginosa infections.

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous Gram-negative opportunistic pathogen and one of the leading causes of healthcare-associated infections worldwide. It is frequently implicated in urinary tract infections, wound infections, ventilator-associated pneumonia, bloodstream infections, and infections in immunocompromised individuals.[1]

One of the most important virulence characteristics of P. aeruginosa is its ability to form biofilms, which are structured microbial communities enclosed within a self-produced extracellular polymeric matrix. Biofilm formation enhances bacterial adherence to biotic and abiotic surfaces, protects bacteria from host immune responses, and markedly reduces the penetration and efficacy of antimicrobial agents. Consequently, biofilm-associated infections are often persistent, recurrent, and difficult to eradicate.[2,3]

The organism possesses remarkable intrinsic and acquired resistance mechanisms, enabling it to survive under diverse environmental conditions and making treatment increasingly challenging.[4,5] In addition to biofilm production, P. aeruginosa expresses a wide range of virulence factors, including

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elastase, protease, phospholipase, pyocyanin, exotoxin A, hemolysin, and motility-associated factors, which contribute to tissue invasion, immune evasion, and disease severity. The coordinated expression of these virulence determinants plays a significant role in bacterial pathogenicity and is often regulated through quorum sensing mechanisms.[6]

The increasing prevalence of multidrug-resistant (MDR) P. aeruginosa has emerged as a major global public health concern. Resistance results from multiple mechanisms, including reduced outer membrane permeability, efflux pumps, β-lactamase production, target-site modifications, and biofilm-mediated protection. Studies have demonstrated a close association between biofilm formation, enhanced virulence, and multidrug resistance, leading to prolonged hospitalization, increased healthcare costs, and higher morbidity and mortality.[5-7]

Understanding the relationship between biofilm production, virulence factors, and multidrug resistance is essential for improving infection control strategies, optimizing antimicrobial therapy, and developing novel therapeutic approaches. Therefore, the present study was undertaken to evaluate the association between biofilm formation, virulence factors, and multidrug resistance among clinical isolates of Pseudomonas aeruginosa obtained from patients attending a tertiary care hospital.

MATERIALS AND METHODS

Study Design: This was a hospital-based cross-sectional analytical study.

Study Setting: The study was conducted in the Department of Microbiology at a tertiary care teaching hospital in collaboration with the Departments of Medicine, Surgery, Intensive Care Unit, and other clinical specialties.

Study Duration: The study was carried out over 6 months.

Sample Size: A total of 160 non-duplicate clinical isolates of Pseudomonas aeruginosa were included in the study.

Study Population: Clinical isolates of Pseudomonas aeruginosa recovered from various clinical specimens submitted to the microbiology laboratory during the study period were included.

Inclusion Criteria

- Non-duplicate clinical isolates of Pseudomonas aeruginosa obtained from patients of all age groups and both sexes.
- Isolates recovered from urine, pus, wound swabs, blood, sputum, endotracheal aspirates, bronchoalveolar lavage, body fluids, catheter tips, ear swabs, and other clinically significant specimens.
- Pure isolates confirmed by standard microbiological identification methods.

Exclusion Criteria

- Duplicate isolates from the same patient.
- Environmental isolates.
- Mixed bacterial growth or contaminated cultures.
- Isolates with incomplete laboratory records.

Isolation and Identification: Clinical specimens were cultured on Blood agar and MacConkey agar and incubated aerobically at 35–37°C for 18–24 hours. Identification of P. aeruginosa was based on colony morphology, characteristic pigment production, grape-like odor, Gram staining, oxidase positivity, motility, growth at 42°C, citrate utilization, oxidative glucose metabolism, and other standard biochemical tests.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to the latest Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotics tested included piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, gentamicin, amikacin, ciprofloxacin, levofloxacin, imipenem, meropenem, and colistin. Multidrug resistance (MDR) was defined as resistance to at least one antimicrobial agent in three or more antimicrobial classes.

Detection of Biofilm Formation: Biofilm production was evaluated by the Tissue Culture Plate (TCP) method, which served as the reference method. Optical density values obtained after crystal violet staining were used to categorize isolates as non-biofilm producers, weak, moderate, or strong biofilm producers.

Detection of Virulence Factors

Each isolate was tested phenotypically for important virulence factors, including:

- Hemolysin production
- Protease production
- Gelatinase activity
- Phospholipase production
- DNase production
- Pyocyanin pigment production

Standard microbiological techniques and appropriate quality-control strains were employed throughout the study.

Outcome Measures: The primary outcome was to determine the prevalence of biofilm formation among Pseudomonas aeruginosa isolates. Secondary outcomes included the prevalence of virulence factors, the frequency of multidrug resistance, and the association between biofilm production, virulence factors, and multidrug resistance.

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using SPSS version 26.0. Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as mean ± standard deviation. Associations between biofilm formation, virulence factors, and multidrug resistance were analyzed using the Chi-square test or Fisher's exact test, as appropriate. Odds ratios with 95% confidence intervals were calculated where applicable. A p-value <0.05 was considered statistically significant.

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RESULTS

A total of 160 non-duplicate clinical isolates of Pseudomonas aeruginosa obtained from various clinical specimens were included in the study. All isolates were evaluated for biofilm formation, virulence factors, and antimicrobial susceptibility. The association between biofilm production, virulence determinants, and multidrug resistance (MDR) was analyzed.

Table 1: Distribution of Pseudomonas aeruginosa Isolates According to Clinical Specimen (n = 160)

Clinical Specimen	Number (n)	Percentage (%)
Pus/Wound swab	54	33.8
Urine	34	21.3
Sputum/Endotracheal aspirate	28	17.5
Blood	18	11.2
Ear swab	14	8.8
Body fluids	12	7.4
Total	160	100

Pus and wound swab specimens constituted the largest proportion of P. aeruginosa isolates (33.8%), followed by urine (21.3%) and respiratory specimens (17.5%).

Table 2: Distribution of Biofilm Formation and Virulence Factors Among Pseudomonas aeruginosa Isolates (n = 160)

Parameter	Number (n)	Percentage (%)
Biofilm Formation		
Non-biofilm producer	38	23.8
Weak biofilm producer	44	27.5
Moderate biofilm producer	46	28.7
Strong biofilm producer	32	20.0
Virulence Factors		
Hemolysin production	118	73.8
Protease production	126	78.8
Phospholipase production	96	60.0
Pyocyanin production	112	70.0
DNase production	74	46.3

Biofilm formation was observed in 122 (76.2%) isolates, with moderate biofilm production being the most frequent category (28.7%). Protease production was the commonest virulence factor (78.8%), followed by hemolysin (73.8%) and pyocyanin production (70.0%).

Table 3: Antimicrobial Resistance Pattern and Multidrug Resistance Among Pseudomonas aeruginosa Isolates (n = 160)

Antibiotic	Resistant n (%)
Ceftazidime	82 (51.3)
Cefepime	76 (47.5)
Ciprofloxacin	70 (43.8)
Gentamicin	64 (40.0)
Amikacin	42 (26.3)
Piperacillin-Tazobactam	48 (30.0)
Imipenem	52 (32.5)
Meropenem	56 (35.0)
Multidrug-resistant (MDR) isolates	72 (45.0)

The highest resistance was observed against ceftazidime (51.3%), followed by cefepime (47.5%) and ciprofloxacin (43.8%). Overall, 45.0% of isolates were classified as multidrug resistant.

Table 4: Association Between Biofilm Formation and Multidrug Resistance (n = 160)

Biofilm Category	MDR Present n (%)	MDR Absent n (%)	Total	p-value
Non-biofilm producer (n=38)	8 (21.1)	30 (78.9)	38	
Weak biofilm producer (n=44)	14 (31.8)	30 (68.2)	44	
Moderate biofilm producer (n=46)	24 (52.2)	22 (47.8)	46	
Strong biofilm producer (n=32)	26 (81.3)	6 (18.7)	32	<0.001

A statistically significant association was observed between biofilm production and multidrug resistance ($p < 0.001$). The proportion of MDR isolates increased progressively with increasing biofilm-forming ability, with 81.3% of strong biofilm producers demonstrating multidrug resistance.

DISCUSSION

The present study evaluated the association between biofilm formation, virulence factors, and multidrug resistance (MDR) among clinical isolates of Pseudomonas aeruginosa. Biofilm formation was

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detected in 76.2% of isolates, with moderate and strong biofilm producers accounting for nearly half of the study population. Furthermore, a significant association was observed between biofilm production and multidrug resistance, indicating that isolates with greater biofilm-forming ability exhibited higher antimicrobial resistance.

The high prevalence of biofilm-producing isolates observed in the present study is consistent with previous reports. Müsken et al. demonstrated that several genetic determinants regulate biofilm establishment in P. aeruginosa, enabling the organism to persist in hostile environments and evade antimicrobial therapy.[8] Similarly, Lee and Yoon reported that biofilm formation represents a highly organized survival strategy that promotes chronic colonization and increases bacterial fitness under adverse conditions.[9] Thi et al. further emphasized that biofilm-associated cells exhibit altered metabolic activity and reduced antibiotic penetration, contributing significantly to treatment failure and recurrent infections.[10]

The present study also demonstrated a high prevalence of virulence factors, particularly protease, hemolysin, and pyocyanin production. These extracellular enzymes and pigments contribute to tissue destruction, immune evasion, and bacterial dissemination. Breidenstein et al. highlighted that the pathogenicity of P. aeruginosa results from the combined action of multiple virulence determinants together with intrinsic and acquired antimicrobial resistance mechanisms.[11] Gellatly and Hancock similarly described that virulence factor expression is tightly regulated through quorum sensing systems, facilitating coordinated host invasion and persistence during infection.[12]

Nearly half of the isolates in the present study were multidrug resistant, and MDR was significantly more common among moderate and strong biofilm producers. This observation supports previous evidence indicating that biofilm formation is closely linked with antimicrobial resistance. Ciofu and Tolker-Nielsen reported that biofilm-associated bacteria develop increased tolerance through restricted antibiotic diffusion, reduced metabolic activity, persister-cell formation, and activation of stress-response pathways, making eradication particularly difficult.[13] Likewise, Botelho et al. emphasized that the global emergence of MDR P. aeruginosa is driven by the combined effects of resistance genes, efflux pumps, β-lactamase production, porin alterations, and adaptive responses associated with biofilm formation.[14]

The significant association between biofilm formation and multidrug resistance observed in the present study has important clinical implications. Horcajada et al. reported that infections caused by MDR and biofilm-producing P. aeruginosa are associated with prolonged hospitalization, limited therapeutic options, increased healthcare expenditure, and higher morbidity and mortality.[15] Early detection of biofilm-producing MDR isolates may therefore aid clinicians in selecting appropriate antimicrobial therapy and implementing effective infection-control measures.

Overall, the findings of the present study reinforce the close relationship between biofilm formation, virulence characteristics, and multidrug resistance in Pseudomonas aeruginosa, highlighting the need for continuous microbiological surveillance and stringent antimicrobial stewardship to reduce the burden of difficult-to-treat infections.

CONCLUSION

Biofilm formation was highly prevalent among clinical isolates of Pseudomonas aeruginosa and showed a significant association with multidrug resistance. Protease, hemolysin, and pyocyanin were the most frequently detected virulence factors. Strong biofilm-producing isolates demonstrated the highest frequency of multidrug resistance, emphasizing the important role of biofilm in bacterial persistence and antimicrobial tolerance. Routine assessment of biofilm formation and virulence characteristics, together with antimicrobial susceptibility testing, can facilitate early identification of high-risk isolates, optimize antimicrobial therapy, and strengthen infection prevention and antimicrobial stewardship programs.

REFERENCES

1. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999 May 21;284(5418):1318-22. doi: 10.1126/science.284.5418.1318.
2. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004 Feb;2(2):95-108. doi: 10.1038/nrmicro821.
3. Bjarnsholt T. The role of bacterial biofilms in chronic infections. APMIS Suppl. 2013 May;(136):1-51. doi: 10.1111/apm.12099.
4. Moradali MF, Ghods S, Rehm BHA. Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol. 2017 Feb 15;7:39. doi: 10.3389/fcimb.2017.00039.
5. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnol Adv. 2019 Jan-Feb;37(1):177-192. doi: 10.1016/j.biotechadv.2018.11.013.
6. Mulcahy LR, Isabella VM, Lewis K. Pseudomonas aeruginosa biofilms in disease. Microb Ecol. 2014 Jan;68(1):1-12. doi: 10.1007/s00248-013-0297-x.
7. Lambert PA. Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. J R Soc Med. 2002;95(Suppl 41):22-26.
8. Müsken M, Di Fiore S, Dötsch A, Fischer R, Häussler S. Genetic determinants of Pseudomonas aeruginosa biofilm establishment. Microbiology. 2010 Feb;156(Pt 2):431-441. doi: 10.1099/mic.0.033290-0.
9. Lee K, Yoon SS. Pseudomonas aeruginosa biofilm, a programmed bacterial life for fitness. J Microbiol Biotechnol. 2017 Jul 28;27(6):1053-1064. doi: 10.4014/jmb.1611.11056.
10. Thi MTT, Wibowo D, Rehm BHA. Pseudomonas aeruginosa biofilms. Int J Mol Sci. 2020 Nov 20;21(22):8671. doi: 10.3390/ijms21228671.
11. Breidenstein EBM, de la Fuente-Núñez C, Hancock REW. Pseudomonas aeruginosa: all roads lead to resistance. Trends Microbiol. 2011 Aug;19(8):419-426. doi: 10.1016/j.tim.2011.04.005.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

12. Gellatly SL, Hancock REW. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathog Dis. 2013 Jul;67(3):159-173. doi: 10.1111/2049-632X.12033.
13. Ciofu O, Tolker-Nielsen T. Tolerance and resistance of Pseudomonas aeruginosa biofilms to antimicrobial agents—How P. aeruginosa can escape antibiotics. Front Microbiol. 2019 May 14;10:913. doi: 10.3389/fmicb.2019.00913.
14. Botelho J, Grosso F, Peixe L. Antibiotic resistance in Pseudomonas aeruginosa—Mechanisms, epidemiology and evolution. Drug Resist Updat. 2019 Nov-Dec;44:100640. doi: 10.1016/j.drup.2019.07.002.
15. Horcajada JP, Montero M, Oliver A, Sorlí L, Luque S, Gómez-Zorrilla S, et al. Epidemiology and treatment of multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa infections. Clin Microbiol Rev. 2019 Mar 13;32(4):e00031-19. doi: 10.1128/CMR.00031-19.

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