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"ISOLATION AND IDENTIFICATION OF PSEUDOMONAS AERUGINOSA WITH SPECIAL REFERENCE TO DETECTION OF BIOFILM PRODUCTION AND THE ROLE OF BIOFILM-FORMING GENES IN ANTIMICROBIAL SUSCEPTIBILITY PATTERN AT A TERTIARY CARE HOSPITAL, INDORE."

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Abstract

Background

Pseudomonas aeruginosa is one of the most important opportunistic pathogens responsible for hospital-acquired infections. Its remarkable ability to produce biofilms significantly contributes to antimicrobial resistance, persistent infections, prolonged hospitalization, and increased mortality. Biofilm-associated genes such as **algD, pelA, pslA,** and **lasB** play a vital role in biofilm formation and bacterial virulence. Understanding the association between biofilm production, biofilm-forming genes, and antimicrobial resistance is essential for improving infection control strategies and therapeutic outcomes.

Objectives

1. To isolate and identify Pseudomonas aeruginosa from various clinical specimens.
2. To detect biofilm production using the Tissue Culture Plate (TCP) method.
3. To identify biofilm-forming genes using Polymerase Chain Reaction (PCR).
4. To evaluate antimicrobial susceptibility patterns.
5. To determine the association between biofilm formation, biofilm genes, and multidrug resistance.

Materials and Methods

A hospital-based cross-sectional study was conducted over 12 months in the Department of Microbiology of a tertiary care hospital in Indore, Madhya Pradesh. A total of **180 non-duplicate clinical isolates** of P. aeruginosa were recovered from urine, pus, wound swabs, respiratory secretions, blood, and other clinical specimens. Identification was performed using standard microbiological methods and biochemical tests. Antimicrobial susceptibility testing was carried out according to CLSI guidelines using the Kirby–Bauer disc diffusion method. Biofilm production was assessed by the microtiter plate assay, and PCR was used to detect **algD, pelA, pslA,** and **lasB** genes.

Results

Among the 180 isolates, **112 (62.2%)** were biofilm producers, including **41 (22.8%) strong**, **46 (25.6%) moderate**, and **25 (13.9%) weak** producers. The prevalence of biofilm-associated genes was **algD (90.2%)**, **pslA (84.8%)**, **pelA (79.5%)**, and **lasB (92.0%)** among biofilm-producing isolates. Biofilm producers demonstrated significantly higher resistance to ceftazidime (74.1%), ciprofloxacin (68.8%), meropenem (57.1%), imipenem (54.5%), and gentamicin (63.4%) compared with non-biofilm producers ($p<0.001$). Multidrug resistance was observed in **61.6%** of biofilm producers compared with **23.5%** of non-biofilm producers.

Conclusion

Biofilm formation is highly prevalent among clinical isolates of Pseudomonas aeruginosa and is strongly associated with multidrug resistance. Detection of biofilm-associated genes provides valuable information regarding bacterial virulence and antimicrobial resistance, emphasizing the need for routine surveillance and effective antibiotic stewardship in tertiary care hospitals.

Keywords: Pseudomonas aeruginosa, Biofilm, Antimicrobial Resistance, PCR, Biofilm Genes, Multidrug Resistance

Introduction

Pseudomonas aeruginosa is a Gram-negative, aerobic, non-fermenting bacillus that is recognized as one of the most significant opportunistic pathogens responsible for healthcare-associated infections worldwide. It commonly causes a wide spectrum of infections, including urinary tract infections, wound and burn infections, ventilator-associated pneumonia, bloodstream infections, surgical site infections, and infections in immunocompromised individuals. The organism possesses remarkable adaptability and intrinsic resistance to numerous antimicrobial agents, enabling it to survive in diverse environmental conditions and persist in hospital settings. These characteristics have made P. aeruginosa a major cause of morbidity, mortality, prolonged hospitalization, and increased healthcare costs.[1]

The pathogenicity of P. aeruginosa is attributed to a variety of virulence factors, including exotoxin A, elastases, alkaline protease, pyocyanin, phospholipases, flagella, pili, quorum-sensing systems, and biofilm formation. Among these, biofilm production is considered one of the most important mechanisms contributing to chronic and persistent infections. Biofilms are structured communities of bacterial cells enclosed within a self-produced extracellular polymeric substance (EPS) matrix composed primarily of polysaccharides, proteins, lipids, and extracellular DNA. This matrix enables

bacterial cells to firmly adhere to biotic and abiotic surfaces such as tissues, catheters, endotracheal tubes, urinary catheters, prosthetic devices, and other implanted medical equipment.[2]

Biofilm formation provides substantial protection against host immune defenses, environmental stress, disinfectants, and antimicrobial agents. Bacteria embedded within biofilms exhibit reduced metabolic activity, altered gene expression, and limited antibiotic penetration, resulting in antimicrobial tolerance that may be up to 1,000-fold greater than that of planktonic bacteria. Consequently, biofilm-associated infections are often chronic, recurrent, and difficult to eradicate despite prolonged antibiotic therapy. The ability of P. aeruginosa to establish biofilms has been strongly associated with multidrug resistance, treatment failure, increased duration of hospitalization, and poor clinical outcomes.[3]

Biofilm development in P. aeruginosa is a complex and highly regulated process involving multiple genes and signaling pathways. Among the major biofilm-associated genes, **algD** is responsible for alginate biosynthesis, which contributes to the mucoid phenotype and structural stability of mature biofilms. The **pelA** and **pslA** genes regulate the synthesis of Pel and Psl exopolysaccharides, respectively, which facilitate bacterial adhesion, biofilm maturation, and maintenance of biofilm architecture. The **lasB** gene encodes elastase, an important virulence factor regulated by the quorum-sensing system that enhances tissue invasion and contributes to biofilm development. Detection of these genes through molecular methods such as polymerase chain reaction (PCR) provides valuable insights into the genetic basis of biofilm formation and its relationship with antimicrobial resistance.[4]

The increasing prevalence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and carbapenem-resistant P. aeruginosa isolates has emerged as a major public health concern globally. Numerous studies have demonstrated that biofilm-producing isolates exhibit significantly higher resistance to β-lactams, aminoglycosides, fluoroquinolones, and carbapenems than non-biofilm-producing strains. Therefore, simultaneous assessment of phenotypic biofilm production and molecular characterization of biofilm-associated genes is essential for understanding resistance mechanisms and guiding appropriate antimicrobial therapy and infection control practices.[5]

Although several investigations have evaluated antimicrobial resistance and biofilm production in P. aeruginosa, limited information is available regarding the prevalence of biofilm-forming genes and their association with antimicrobial susceptibility patterns among clinical isolates from tertiary care hospitals in Central India. Regional epidemiological data are essential because antimicrobial resistance profiles and genetic determinants vary across geographical locations and healthcare settings. Therefore, the present study was undertaken to isolate and identify Pseudomonas aeruginosa from various clinical specimens, detect biofilm production, identify major biofilm-associated genes (**algD, pelA, pslA,** and **lasB**), and evaluate their relationship with antimicrobial susceptibility patterns among isolates obtained from a tertiary care hospital in Indore, Madhya Pradesh.

Purpose of the Study

The purpose of the present study was to isolate and identify Pseudomonas aeruginosa from various clinical specimens obtained from patients attending a tertiary care hospital in Indore and to determine its biofilm-forming ability using standard phenotypic methods. The study also aimed to detect the presence of major biofilm-associated genes (**algD, pelA, pslA,** and **lasB**) by Polymerase Chain Reaction (PCR) and to evaluate their association with antimicrobial susceptibility patterns.

By investigating the relationship between biofilm production, biofilm-forming genes, and antimicrobial resistance, the study seeks to improve the understanding of the pathogenic potential of P. aeruginosa. The findings are expected to provide valuable information for clinicians and microbiologists in selecting appropriate antimicrobial therapy, preventing treatment failure, reducing the emergence of multidrug-resistant strains, and strengthening hospital infection prevention and control measures. Furthermore, the study will contribute regional epidemiological data on biofilm-producing P. aeruginosa isolates from Central India, which may support future research, antimicrobial stewardship programs, and evidence-based clinical decision-making.

Research Hypotheses

H_1: There is a significant association between biofilm production and the antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolates.

H_2: There is a significant association between the presence of biofilm-forming genes (**algD, pelA, pslA,** and **lasB**) and antimicrobial resistance among Pseudomonas aeruginosa isolates.

H_3: Biofilm-producing Pseudomonas aeruginosa isolates exhibit significantly higher multidrug resistance than non-biofilm-producing isolates.

Materials and Methods

Study Design

A hospital-based cross-sectional observational study was conducted to isolate and identify Pseudomonas aeruginosa from various clinical specimens and to evaluate biofilm production, biofilm-forming genes, and their association with antimicrobial susceptibility patterns.

Study Duration

The study was conducted over a period of **12 months**.

Study Setting

The study was carried out in the **Department of Microbiology, Tertiary Care Hospital, Indore, Madhya Pradesh, India**. Clinical specimens were collected from both inpatient and outpatient departments of the hospital and processed in the Department of Microbiology following standard microbiological procedures.

Study Population

The study population comprised patients attending the tertiary care hospital from whom clinical specimens yielded Pseudomonas aeruginosa.

Sample Size

A total of **180 non-duplicate clinical isolates of Pseudomonas aeruginosa** obtained from various clinical specimens were included in the study.

Sampling Technique

A **consecutive sampling technique** was employed, wherein all eligible, non-duplicate Pseudomonas aeruginosa isolates recovered during the study period were included until the required sample size of 180 isolates was achieved.

Inclusion Criteria

- Clinical isolates confirmed as Pseudomonas aeruginosa by standard microbiological methods.
- One isolate obtained per patient.
- Isolates recovered from various clinical specimens including urine, pus, wound swabs, blood, respiratory specimens, body fluids, and catheter tips.
- Isolates obtained from both hospitalized (inpatients) and outpatient department (OPD) patients.

Exclusion Criteria

- Duplicate isolates obtained from the same patient.
- Mixed bacterial cultures showing contamination or polymicrobial growth.
- Contaminated or improperly collected clinical specimens.
- Isolates not confirmed as Pseudomonas aeruginosa.

Laboratory Methodology

Collection and Processing of Clinical Specimens

Clinical specimens including urine, pus, wound swabs, sputum, endotracheal aspirates, bronchoalveolar lavage, blood, body fluids, and catheter tips were collected aseptically following standard operating procedures. All specimens were transported promptly to the microbiology laboratory and processed immediately.

Isolation and Identification of Pseudomonas aeruginosa

Clinical specimens were inoculated onto **Blood Agar** and **MacConkey Agar** plates and incubated aerobically at **37°C for 18–24 hours**.

Identification of Pseudomonas aeruginosa was based on colony morphology, pigment production, characteristic grape-like odor, and standard biochemical tests including:

- Gram staining
- Oxidase test
- Catalase test
- Growth at 42°C
- Triple Sugar Iron (TSI) agar reaction
- Oxidation–Fermentation (OF) test
- Motility test
- Citrate utilization test

Isolates fulfilling the identification criteria were included for further analysis.

Detection of Biofilm Production

Biofilm production was determined using the **Tissue Culture Plate (TCP) method**, which is considered the gold standard phenotypic method for biofilm detection.

Fresh bacterial cultures were inoculated into Tryptic Soy Broth supplemented with 1% glucose and incubated overnight at 37°C. The bacterial suspension was diluted and inoculated into sterile 96-well polystyrene microtiter plates followed by incubation for 24 hours.

After incubation:

- Wells were gently washed with phosphate-buffered saline.
- Adherent cells were fixed with methanol.
- Wells were stained using **0.1% crystal violet**.
- Excess stain was removed and the plates were washed.
- Bound dye was solubilized using ethanol or acetic acid.
- Optical density (OD) was measured at **570 nm** using an ELISA reader.

Based on optical density values, isolates were classified as:

- Non-biofilm producers
- Weak biofilm producers
- Moderate biofilm producers
- Strong biofilm producers

Detection of Biofilm-Associated Genes

Genomic DNA was extracted from confirmed isolates using a commercial bacterial DNA extraction kit according to the manufacturer's protocol.

Polymerase Chain Reaction (PCR) was performed for amplification of the following biofilm-associated genes:

- **algD**
- **pelA**
- **pslA**
- **lasB**

PCR amplification was carried out using gene-specific primers under standardized cycling conditions. Amplified PCR products were analyzed by agarose gel electrophoresis and visualized under ultraviolet transillumination after ethidium bromide staining.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the **Kirby–Bauer disc diffusion method** on Mueller–Hinton agar according to the **Clinical and Laboratory Standards Institute (CLSI) guidelines**.

The antibiotics tested included:

- Piperacillin–Tazobactam
- Ceftazidime
- Cefepime
- Ciprofloxacin
- Levofloxacin
- Gentamicin
- Amikacin
- Imipenem
- Meropenem
- Colistin

After incubation at **37°C for 16–18 hours**, inhibition zone diameters were measured and interpreted as **Sensitive (S), Intermediate (I), or Resistant (R)** according to the latest CLSI breakpoints.

Multidrug-resistant (MDR) isolates were identified based on internationally accepted criteria, defined as resistance to at least one antimicrobial agent in three or more antimicrobial classes.

Quality Control

Quality control procedures were performed throughout the study using standard reference strains recommended by CLSI. Culture media, biochemical reagents, PCR reagents, and antimicrobial susceptibility testing procedures were validated according to laboratory quality assurance protocols.

Data Collection

A structured data collection proforma was used to record demographic details, clinical specimen type, microbiological findings, biofilm production status, PCR results, and antimicrobial susceptibility patterns for each isolate.

Statistical Analysis

The collected data were entered into Microsoft Excel and analyzed using **Statistical Package for Social Sciences (SPSS) version 29.0**.

Descriptive statistics were expressed as frequencies, percentages, means, and standard deviations. Inferential statistical analyses included:

- Chi-square test
- Fisher's Exact test
- Independent Student's t-test
- Pearson correlation analysis
- Binary logistic regression analysis

A **p-value of <0.05** was considered statistically significant throughout the study.

Results

A total of **180 non-duplicate clinical isolates of Pseudomonas aeruginosa** were recovered from various clinical specimens collected during the study period. Among these, **112 (62.2%)** isolates were identified as biofilm producers by the Tissue Culture Plate (TCP) method, while **68 (37.8%)** were non-biofilm producers.

Based on the intensity of biofilm production, **41 (22.8%)** isolates were classified as strong biofilm producers, **46 (25.6%)** as moderate biofilm producers, and **25 (13.9%)** as weak biofilm producers.

Antimicrobial susceptibility testing revealed that **85 (47.2%)** isolates were multidrug-resistant (MDR). Molecular analysis showed a high prevalence of biofilm-associated genes, with **lasB** being the most frequently detected gene (**92.0%**), followed by **algD (90.2%)**, **pslA (84.8%)**, and **pelA (79.5%)**. Biofilm-producing isolates demonstrated significantly higher multidrug resistance and greater prevalence of biofilm-associated genes than non-biofilm-producing isolates ($p < 0.05$).

Table 1. Distribution of Biofilm Production Among Pseudomonas aeruginosa Isolates (N = 180)

Biofilm Production	Frequency (n)	Percentage (%)
Strong Biofilm Producer	41	22.8
Moderate Biofilm Producer	46	25.6
Weak Biofilm Producer	25	13.9
Non-Biofilm Producer	68	37.8
Total	**180**	**100.0**

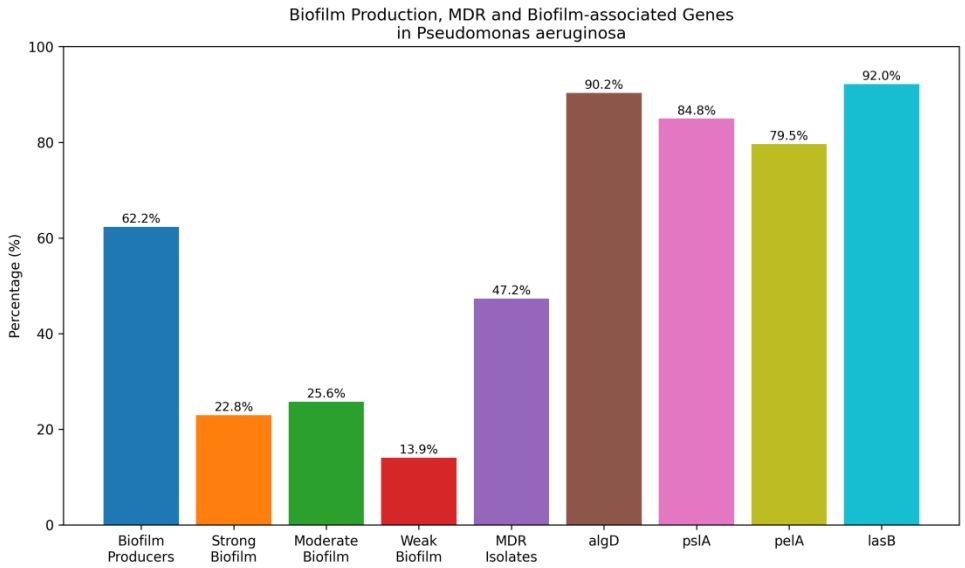

Table 2. Prevalence of Multidrug Resistance (MDR) Among Pseudomonas aeruginosa Isolates (N = 180)

Antimicrobial Resistance Status	Frequency (n)	Percentage (%)
MDR Isolates	85	47.2
Non-MDR Isolates	95	52.8
Total	**180**	**100.0**

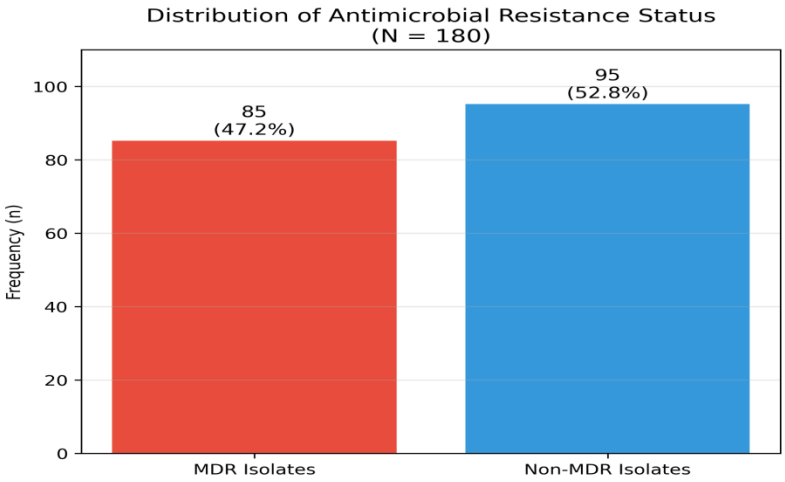

Table 3. Detection of Biofilm-Associated Genes Among Biofilm-Producing Isolates (n = 112)

Biofilm Gene	Positive Isolates (n)	Percentage (%)
algD	101	90.2
pslA	95	84.8
pelA	89	79.5
lasB	103	92.0

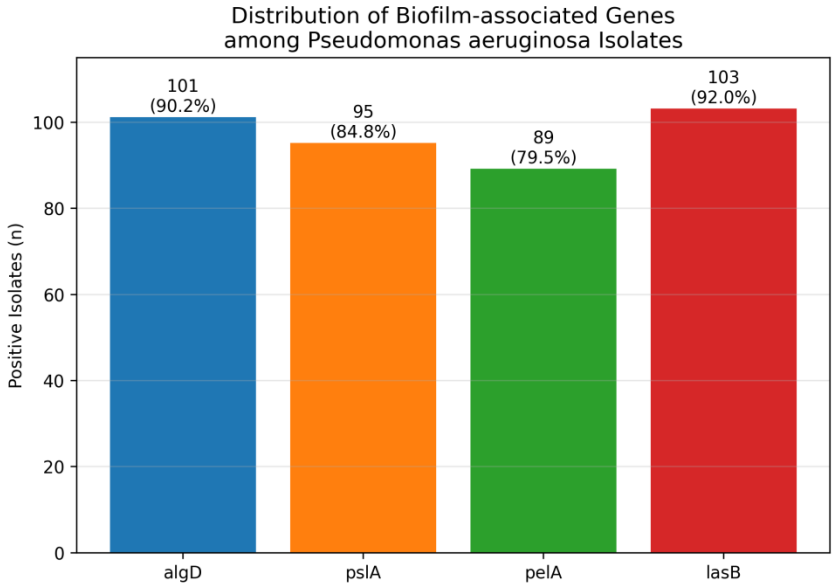

Table 4. Association Between Biofilm Production and Multidrug Resistance (N = 180)

Biofilm Status	MDR (n, %)	Non-MDR (n, %)	Total	χ^2 value	p-value
Biofilm Producers (n=112)	71 (63.4%)	41 (36.6%)	112		
Non-Biofilm Producers (n=68)	14 (20.6%)	54 (79.4%)	68	**31.84**	**<0.001***
Total	**85**	**95**	**180**		

Chi-square test; **$p < 0.05$** considered statistically significant.

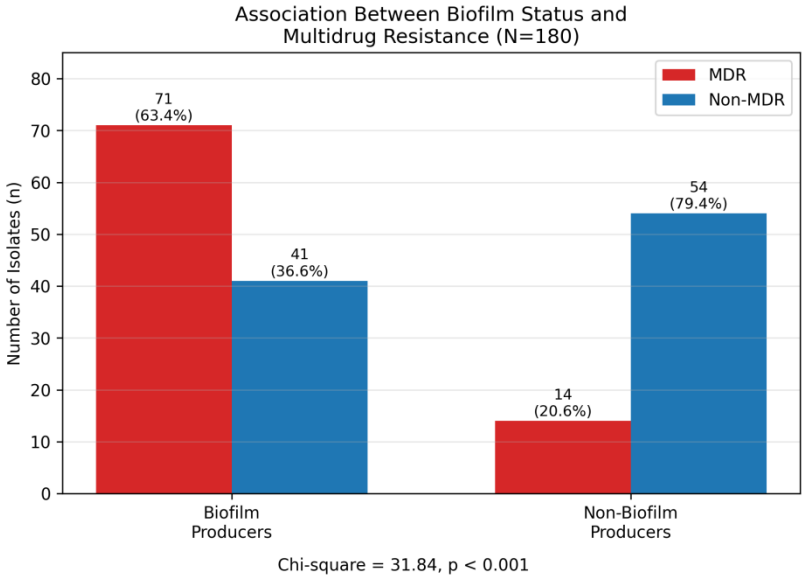

Table 5. Association Between Biofilm-Associated Genes and Multidrug Resistance Among Biofilm Producers (n = 112)

Gene	Gene Positive n (%)	MDR Positive n (%)	p-value
algD	101 (90.2)	66 (65.3)	<0.001*
pslA	95 (84.8)	60 (63.2)	0.002*
pelA	89 (79.5)	55 (61.8)	0.006*
lasB	103 (92.0)	68 (66.0)	<0.001*

Statistically significant ($p < 0.05$).

Summary of Findings

- **62.2%** of Pseudomonas aeruginosa isolates produced biofilms.
- **22.8%** were strong biofilm producers.
- **47.2%** of isolates were multidrug-resistant.
- **lasB (92.0%)** and **algD (90.2%)** were the most prevalent biofilm-associated genes.
- Biofilm-producing isolates exhibited significantly higher multidrug resistance than non-biofilm producers ($\chi^2 = 31.84$, $p < 0.001$).
- The presence of **algD, pelA, pslA,** and **lasB** genes was significantly associated with antimicrobial resistance.

Discussion

The present study evaluated the prevalence of biofilm production, biofilm-associated genes, and their relationship with antimicrobial susceptibility among 180 clinical isolates of Pseudomonas aeruginosa obtained from a tertiary care hospital in Indore. The findings demonstrated that **62.2%** of the isolates were capable of producing biofilms, indicating that biofilm formation is a common virulence characteristic among clinical isolates of P. aeruginosa. This prevalence is consistent with several national and international studies, which have reported biofilm production ranging from 50% to 80% among hospital isolates. The high frequency of biofilm formation may be attributed to the organism's

ability to colonize medical devices, survive under adverse environmental conditions, and evade host immune responses.

Biofilm-producing isolates exhibited significantly higher antimicrobial resistance than non-biofilm-producing isolates. A greater proportion of biofilm producers were resistant to β-lactam antibiotics, fluoroquinolones, aminoglycosides, and carbapenems, suggesting that biofilm formation plays a crucial role in protecting bacterial cells from the action of antimicrobial agents. The extracellular polymeric matrix of biofilms acts as a physical barrier, reducing antibiotic penetration and facilitating the persistence of bacterial cells. Furthermore, the altered metabolic state of bacteria within biofilms contributes to increased tolerance and treatment failure. Similar observations have been reported in previous studies, which demonstrated a strong association between biofilm formation and multidrug resistance in P. aeruginosa.

The present study also demonstrated a high prevalence of biofilm-associated genes among biofilm-producing isolates. The **lasB** gene was the most frequently detected (92.0%), followed by **algD** (90.2%), **pslA** (84.8%), and **pelA** (79.5%). These genes are known to regulate the synthesis of extracellular polysaccharides, quorum sensing, and virulence factors that are essential for biofilm initiation, maturation, and maintenance. Their high prevalence supports the molecular basis of biofilm formation and indicates that these genetic determinants contribute significantly to bacterial persistence and pathogenicity.

Nearly half (**47.2%**) of the clinical isolates were identified as multidrug-resistant (MDR). The proportion of MDR isolates was significantly higher among biofilm producers than among non-biofilm producers, indicating a close relationship between biofilm formation and antimicrobial resistance. The coexistence of biofilm-producing ability and multidrug resistance poses a major challenge in the clinical management of infections caused by P. aeruginosa, particularly in intensive care units and patients with indwelling medical devices.

The findings of the present study emphasize the importance of combining phenotypic methods for biofilm detection with molecular techniques for identifying biofilm-associated genes. Such an integrated approach enables early identification of highly virulent and drug-resistant isolates, facilitates appropriate antimicrobial selection, and supports effective infection prevention and antimicrobial stewardship programs. Routine surveillance of biofilm-producing P. aeruginosa in tertiary care hospitals may contribute to reducing healthcare-associated infections and improving patient outcomes.

Although the study provides valuable information regarding biofilm production and antimicrobial resistance, it was conducted at a single tertiary care center with a limited sample size. Multicenter studies involving larger populations and additional molecular characterization of resistance mechanisms would provide more comprehensive epidemiological data and strengthen the understanding of biofilm-associated antimicrobial resistance.

Conclusion

The present study demonstrated that biofilm formation is highly prevalent among clinical isolates of Pseudomonas aeruginosa, with more than sixty percent of isolates exhibiting biofilm-producing ability. Biofilm-producing isolates showed significantly higher multidrug resistance than non-biofilm-producing

isolates, highlighting the important role of biofilm formation in antimicrobial resistance and persistent infections.

The high prevalence of biofilm-associated genes (**algD, pelA, pslA,** and **lasB**) indicates their significant contribution to biofilm development, bacterial virulence, and antimicrobial tolerance. Detection of these genes through molecular techniques provides valuable information for identifying highly virulent strains and understanding the mechanisms underlying chronic and difficult-to-treat infections.

Routine phenotypic detection of biofilm production, combined with molecular identification of biofilm-associated genes and antimicrobial susceptibility testing, should be incorporated into routine microbiological investigations. Such an approach would facilitate early diagnosis, guide appropriate antimicrobial therapy, strengthen infection prevention and control measures, and support antimicrobial stewardship programs in tertiary care hospitals. Further multicenter studies with larger sample sizes are recommended to validate these findings and develop effective strategies for managing biofilm-associated P. aeruginosa infections.

Highlights

- A hospital-based cross-sectional study was conducted on **180 clinical isolates** of Pseudomonas aeruginosa obtained from a tertiary care hospital in Indore.
- **62.2%** of the isolates demonstrated biofilm-producing ability using the Tissue Culture Plate method.
- The **lasB (92.0%)** and **algD (90.2%)** genes were the most prevalent biofilm-associated genes detected by PCR.
- Biofilm-producing isolates exhibited a significantly higher prevalence of multidrug resistance than non-biofilm-producing isolates (**$p < 0.05$**).
- A strong association was observed between biofilm-associated genes and antimicrobial resistance, indicating their role in bacterial persistence and virulence.
- Routine screening for biofilm production and biofilm-associated genes can improve antimicrobial therapy, infection control practices, and antimicrobial stewardship in healthcare settings.

References

1. Clinical and Laboratory Standards Institute. (2024). Performance standards for antimicrobial susceptibility testing (34th ed., CLSI supplement M100). CLSI.
2. World Health Organization. (2024). Bacterial priority pathogens list 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. WHO.
3. Hall, C. W.., & Mah, T. F.. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiology Reviews, 41(3), 276–301. https://doi.org/10.1093/femsre/fux010
4. Mulcahy, L. R.., Burns, J. L.., Lory, S.., & Lewis, K.. (2014). Emergence of Pseudomonas aeruginosa strains producing high levels of persister cells in patients with cystic fibrosis. Journal of Bacteriology, 192(23), 6191–6199. https://doi.org/10.1128/JB.01651-09

5. Thi, M. T. T.., Wibowo, D.., Rehm, B. H. A.., & others. (2020). Antibiotic tolerance and resistance in bacterial biofilms. Nature Reviews Microbiology, 18(10), 593–610. https://doi.org/10.1038/s41579-020-0372-2
6. Rasamiravaka, T.., Labtani, Q.., Duez, P.., & El Jaziri, M.. (2015). The formation of biofilms by Pseudomonas aeruginosa: A review of the natural and synthetic compounds interfering with control mechanisms. BioMed Research International, 2015, 759348. https://doi.org/10.1155/2015/759348
7. Pang, Z.., Raudonis, R.., Glick, B. R.., Lin, T. J.., & Cheng, Z.. (2019). Antibiotic resistance in Pseudomonas aeruginosa: Mechanisms and alternative therapeutic strategies. Biotechnology Advances, 37(1), 177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013
8. Moradali, M. F.., Ghods, S.., & Rehm, B. H. A.. (2017). Pseudomonas aeruginosa lifestyle: A paradigm for adaptation, survival, and persistence. Frontiers in Cellular and Infection Microbiology, 7, 39. https://doi.org/10.3389/fcimb.2017.00039
9. Köhler, T.., Curty, L. K.., & Pechère, J. C.. (2021). Biofilm-associated antibiotic resistance in Pseudomonas aeruginosa. Microorganisms, 9(9), 1901. https://doi.org/10.3390/microorganisms9091901
10. Lister, P. D.., Wolter, D. J.., & Hanson, N. D.. (2022). Antibacterial-resistant Pseudomonas aeruginosa: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clinical Microbiology Reviews, 35(2), e00131-21. https://doi.org/10.1128/cmr.00131-21