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"ANTIMICROBIAL RESISTANCE PATTERNS AND BIOFILM-PRODUCING ABILITY OF CLINICAL PSEUDOMONAS AERUGINOSA ISOLATES: A HOSPITAL-BASED CROSS-SECTIONAL STUDY."

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Abstract

Background

Pseudomonas aeruginosa is an important opportunistic pathogen responsible for healthcare-associated infections and is recognized for its intrinsic antimicrobial resistance and remarkable ability to produce biofilms. Biofilm formation enhances bacterial survival, contributes to persistent infections, and reduces the effectiveness of antimicrobial therapy. Understanding the relationship between biofilm production and antimicrobial resistance is essential for improving infection control and patient management.

Objectives

To determine the antimicrobial resistance patterns and biofilm-producing ability of clinical *Pseudomonas aeruginosa* isolates and to evaluate the association between biofilm formation and multidrug resistance in a tertiary care hospital.

Materials and Methods

A hospital-based cross-sectional study was conducted over 12 months in the Department of Microbiology of a tertiary care hospital. A total of **180 non-duplicate clinical isolates** of *Pseudomonas aeruginosa* recovered from urine, pus, wound swabs, sputum, blood, endotracheal aspirates, and other clinical specimens were included. Identification was performed using standard microbiological methods. Antimicrobial susceptibility testing was carried out by the Kirby–Bauer disc diffusion method according to CLSI guidelines. Biofilm production was detected using the Tissue Culture Plate (TCP) method. Statistical analysis was performed using SPSS version 29.0, and a p-value <0.05 was considered statistically significant.

Results

Among the 180 isolates, **112 (62.2%)** were identified as biofilm producers, while **68 (37.8%)** were non-biofilm producers. Strong biofilm production was observed in **22.8%**, moderate in **25.6%**, and weak in **13.9%** of isolates. Overall, **85 (47.2%)** isolates were multidrug resistant (MDR). Biofilm-producing isolates exhibited significantly higher resistance to ceftazidime, cefepime, ciprofloxacin, gentamicin, amikacin, imipenem, and meropenem compared with non-biofilm producers. A statistically significant association was observed between biofilm production and multidrug resistance ($\chi^2 = 31.84$, $p < 0.001$).

Conclusion

Biofilm formation was highly prevalent among clinical *Pseudomonas aeruginosa* isolates and was significantly associated with multidrug resistance. Routine screening for biofilm production along with antimicrobial susceptibility testing may facilitate early identification of high-risk isolates, optimize antimicrobial therapy, and strengthen infection control practices.

Keywords: *Pseudomonas aeruginosa*, Biofilm, Antimicrobial resistance, Multidrug resistance, Hospital-acquired infection.

1. Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative, aerobic, non-fermenting bacillus that has emerged as one of the most significant causes of healthcare-associated infections worldwide. It is responsible for a wide spectrum of infections, including urinary tract infections, wound and burn wound infections, pneumonia, ventilator-associated pneumonia, bloodstream infections, surgical site infections, otitis externa, and infections associated with indwelling medical devices. The organism is particularly associated with immunocompromised individuals, intensive care unit (ICU) patients, and those with chronic underlying diseases, where it contributes substantially to morbidity, mortality, prolonged hospitalization, and increased healthcare costs. Its remarkable ability to survive under adverse environmental conditions and acquire multiple resistance mechanisms makes *P. aeruginosa* one of the most challenging pathogens encountered in clinical practice.

A major factor contributing to the pathogenicity of *P. aeruginosa* is its extensive repertoire of virulence determinants, including exotoxin A, elastase, alkaline protease, pyocyanin, phospholipase C, flagella, pili, and quorum-sensing systems. Among these virulence factors, biofilm formation is considered one of the most important mechanisms responsible for persistent and chronic infections. Biofilms are highly organized microbial communities enclosed within a self-produced extracellular polymeric substance (EPS) composed primarily of polysaccharides, proteins, extracellular DNA, and lipids. These microbial communities firmly adhere to biotic tissues and abiotic surfaces such as urinary catheters, central venous catheters, endotracheal tubes, orthopedic implants, prosthetic heart valves, and other medical devices. The biofilm matrix provides structural stability while protecting bacterial cells from host immune responses, desiccation, disinfectants, and antimicrobial agents, thereby facilitating long-term persistence within the host.

Biofilm-associated bacteria exhibit physiological characteristics that differ substantially from their planktonic counterparts. Cells embedded within biofilms display reduced metabolic activity, altered gene expression, and increased tolerance to antimicrobial agents, often requiring antibiotic concentrations many times higher than those effective against free-living bacteria. The extracellular polymeric matrix also limits antibiotic penetration, promotes horizontal gene transfer, and facilitates the exchange of antimicrobial resistance determinants. Consequently, biofilm-associated infections are frequently chronic, recurrent, and refractory to conventional antimicrobial therapy, often necessitating prolonged treatment, surgical intervention, or removal of infected medical devices.

The rapid emergence and dissemination of multidrug-resistant (MDR) *P. aeruginosa* have become major global public health concerns. The organism possesses numerous intrinsic, acquired, and adaptive resistance mechanisms, including production of β-lactamases, carbapenemases, aminoglycoside-modifying enzymes, overexpression of multidrug efflux pumps, decreased outer membrane permeability due to porin loss, target-site mutations, and biofilm-mediated adaptive resistance. These mechanisms frequently coexist within the same isolate, resulting in limited therapeutic options and increased rates of treatment failure. The World Health Organization (WHO) has identified carbapenem-resistant *P. aeruginosa* as one of the priority pathogens requiring urgent research and development of new antimicrobial agents, highlighting its growing clinical significance.

Biofilm formation has been increasingly recognized as an important contributor to antimicrobial resistance in *P. aeruginosa*. Numerous studies have demonstrated that biofilm-producing isolates exhibit significantly higher resistance to β-lactams, fluoroquinolones, aminoglycosides, and carbapenems compared with non-biofilm-producing isolates. Therefore, simultaneous assessment of biofilm-producing ability and antimicrobial susceptibility has become an important component of clinical microbiology laboratories for predicting treatment outcomes and guiding antimicrobial stewardship programs.

Continuous surveillance of antimicrobial resistance patterns is essential for monitoring local epidemiological trends, optimizing empirical antimicrobial therapy, preventing the spread of resistant strains, and developing effective infection prevention and control policies. Hospital-based surveillance studies also provide valuable information for antimicrobial stewardship initiatives and help clinicians make evidence-based therapeutic decisions.

Although several studies have investigated antimicrobial resistance and biofilm production in *P. aeruginosa*, comprehensive data describing their association remain limited in many regions of India, particularly in Central India. Regional differences in antimicrobial prescribing practices, infection control measures, and circulating bacterial strains may significantly influence resistance profiles and biofilm prevalence. Therefore, local epidemiological data are essential for developing region-specific treatment guidelines and infection control strategies.

In this context, the present hospital-based cross-sectional study was undertaken to determine the antimicrobial resistance patterns and biofilm-producing ability of clinical *Pseudomonas aeruginosa* isolates obtained from patients attending a tertiary care hospital. The study also aimed to evaluate the association between biofilm formation and multidrug resistance, thereby providing clinically relevant information that may contribute to improved patient management, rational antimicrobial use, infection prevention practices, and future surveillance of healthcare-associated infections.

Purpose of the Study

Pseudomonas aeruginosa is one of the most important opportunistic pathogens responsible for a wide range of healthcare-associated infections and is increasingly associated with multidrug resistance and treatment failure. Its ability to produce biofilms enhances bacterial survival by protecting the organism from host immune defenses and reducing the efficacy of antimicrobial agents, thereby contributing to persistent and recurrent infections. The growing prevalence of multidrug-resistant (MDR) *P. aeruginosa* has become a major challenge for clinicians due to limited therapeutic options, prolonged hospital stays, increased healthcare costs, and higher patient morbidity and mortality.

Understanding the relationship between biofilm production and antimicrobial resistance is essential for improving the diagnosis and management of *P. aeruginosa* infections. Although several studies have reported the occurrence of biofilm-producing isolates, regional data on their prevalence and antimicrobial resistance patterns remain limited, particularly in tertiary care hospitals of Central India. Continuous monitoring of antimicrobial susceptibility profiles and biofilm-forming ability is necessary to identify emerging resistance trends, optimize empirical antibiotic therapy, strengthen infection prevention strategies, and support antimicrobial stewardship programs.

Therefore, the present hospital-based cross-sectional study was undertaken to isolate and identify clinical *Pseudomonas aeruginosa* isolates, determine their antimicrobial resistance patterns, assess their biofilm-producing ability using phenotypic methods, and evaluate the association between biofilm formation and multidrug resistance. The findings of this study are expected to provide valuable epidemiological information that will assist clinicians in selecting appropriate antimicrobial therapy, enhance infection control practices, and contribute to the development of evidence-based hospital antibiotic policies.

2. Objectives

1. To isolate and identify *Pseudomonas aeruginosa* from clinical specimens.
2. To determine the prevalence of biofilm-producing isolates.
3. To evaluate antimicrobial susceptibility patterns using CLSI guidelines.
4. To determine the prevalence of multidrug-resistant isolates.
5. To assess the association between biofilm production and multidrug resistance.
6. To compare antimicrobial susceptibility between biofilm-producing and non-biofilm-producing isolates.

3. Hypotheses

H_0: There is no significant association between biofilm production and antimicrobial resistance among clinical *Pseudomonas aeruginosa* isolates.

H_1: Biofilm-producing *Pseudomonas aeruginosa* isolates exhibit significantly higher antimicrobial resistance than non-biofilm-producing isolates.

4. Materials and Methods

Study Design

A hospital-based cross-sectional observational study was conducted to evaluate the antimicrobial resistance patterns and biofilm-producing ability of clinical *Pseudomonas aeruginosa* isolates obtained from patients attending a tertiary care hospital. The study was designed to determine the prevalence of biofilm-producing isolates and to assess the association between biofilm formation and antimicrobial resistance.

Study Duration

The study was carried out over a period of **12 months**, from **January 2025 to December 2025**.

Study Setting

The research was conducted in the **Department of Microbiology** of a tertiary care teaching hospital in **Indore, Madhya Pradesh, India**. The microbiology laboratory receives a wide variety of clinical specimens from inpatient departments, intensive care units (ICUs), outpatient departments, emergency services, and specialized care units.

Study Population

The study population comprised patients of all age groups and both sexes who had culture-positive clinical specimens yielding *Pseudomonas aeruginosa*. Only one isolate per patient was included in the study to avoid duplication.

Sample Size

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

Sampling Technique

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

Inclusion Criteria

- Clinical specimens yielding pure growth of *Pseudomonas aeruginosa*.
- One isolate per patient.
- Isolates obtained from both hospitalized and outpatient individuals.
- Isolates recovered during the study period.

Exclusion Criteria

- Duplicate isolates from the same patient.
- Mixed bacterial cultures.
- Contaminated specimens.
- Inadequate or improperly transported specimens.

Collection of Clinical Specimens

Clinical specimens were collected aseptically by trained healthcare personnel according to standard operating procedures. The specimens included:

- Urine
- Pus
- Wound swabs
- Sputum
- Blood
- Endotracheal aspirates
- Bronchoalveolar lavage (BAL)
- Body fluids
- Catheter tips
- Other clinically relevant specimens

Specimens were transported immediately to the microbiology laboratory and processed without delay.

Isolation and Identification of *Pseudomonas aeruginosa*

Clinical specimens were cultured on Blood agar and MacConkey agar plates and incubated aerobically at **37°C for 18–24 hours**. Suspected colonies were identified based on colony morphology, pigment production, characteristic grape-like odor, and standard microbiological techniques.

Confirmation of *Pseudomonas aeruginosa* was performed using:

- Gram staining
- Oxidase test
- Catalase test
- Growth at **42°C**
- Motility test
- Oxidative-Fermentative (OF) glucose test
- Triple Sugar Iron (TSI) agar reaction
- Citrate utilization test
- Other relevant biochemical tests as required

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the **Kirby–Bauer disc diffusion method** on Mueller–Hinton agar following the recommendations of the **Clinical and Laboratory Standards Institute (CLSI) M100, 34th edition**.

The antibiotics tested included:

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

After incubation at **35 ± 2°C for 16–18 hours**, inhibition zone diameters were measured and interpreted as **susceptible, intermediate, or resistant** according to CLSI criteria.

Definition of Multidrug Resistance

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* was defined as resistance to **at least one antimicrobial agent in three or more different classes of antibiotics**, according to internationally accepted criteria.

Detection of Biofilm Production

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

Each isolate was inoculated into **Tryptic Soy Broth (TSB)** supplemented with **1% glucose** and incubated at **37°C for 24 hours**. The cultures were diluted and transferred into sterile 96-well flat-bottom microtiter plates, followed by incubation for another 24 hours.

The wells were gently washed three times with phosphate-buffered saline (PBS) to remove non-adherent bacteria and air-dried. Adherent cells were fixed with methanol, stained with **0.1% crystal violet**, washed thoroughly with distilled water, and allowed to dry. The bound dye was solubilized using **33% glacial acetic acid**, and the optical density (OD) was measured at **570 nm** using a microplate reader.

Based on the OD values, isolates were categorized as:

- Non-biofilm producer
- Weak biofilm producer
- Moderate biofilm producer
- Strong biofilm producer

Quality Control

Quality control procedures were followed throughout the study. Standard reference strain ***Pseudomonas aeruginosa*** **ATCC 27853** was used for antimicrobial susceptibility testing according to CLSI recommendations. All culture media, reagents, and antibiotic discs were quality checked before use.

Data Collection

Data regarding patient demographics, clinical specimen type, culture findings, antimicrobial susceptibility results, and biofilm production status were recorded using a structured data collection proforma.

Statistical Analysis

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

Descriptive statistics were expressed as frequencies, percentages, means, and standard deviations. The association between biofilm production and antimicrobial resistance was assessed using the **Chi-square test** or **Fisher's exact test**, wherever appropriate. Continuous variables were compared using the **independent Student's t-test**. A **p-value < 0.05** was considered statistically significant.

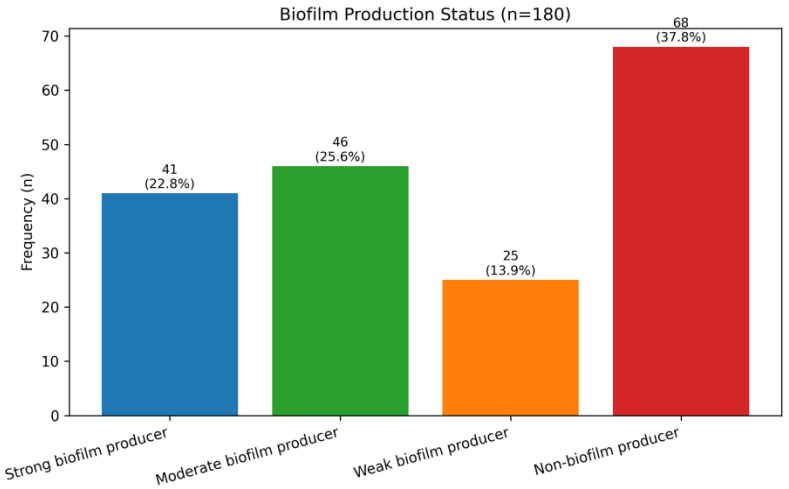
Ethical Considerations

The study protocol was reviewed and approved by the **Institutional Ethics Committee (IEC)** of the participating institution before commencement of the study. Permission was obtained from the hospital authorities and the Department of Microbiology. Patient confidentiality was maintained by anonymizing all clinical and laboratory data, and the study adhered to the ethical principles outlined in the **Declaration of Helsinki**.

5. Results

Table 1. Distribution of Biofilm-Producing *Pseudomonas aeruginosa* Isolates (N = 180)

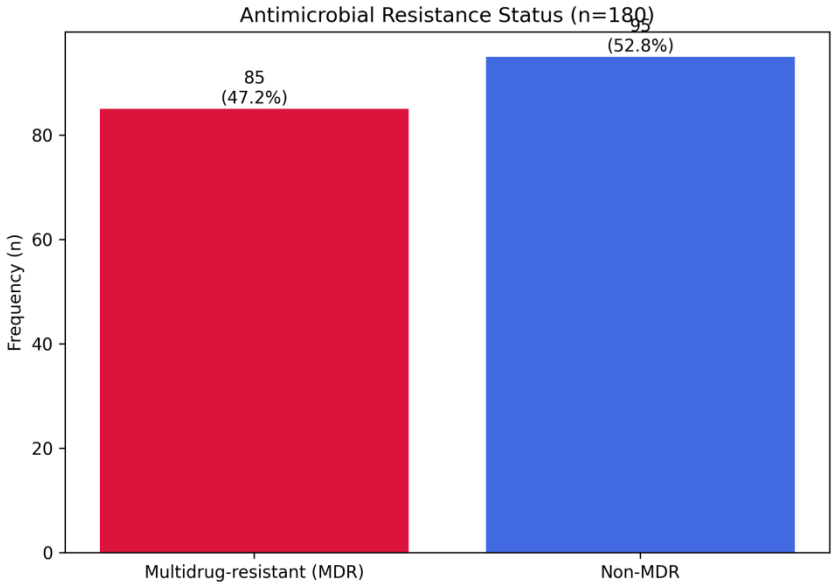
Biofilm Production Status	Frequency (n)	Percentage (%)
Strong biofilm producer	41	22.8
Moderate biofilm producer	46	25.6
Weak biofilm producer	25	13.9
Non-biofilm producer	68	37.8
Total	**180**	**100.0**



Interpretation:
Table 1 shows the distribution of biofilm-producing *Pseudomonas aeruginosa* isolates. Among the 180 isolates, **112 (62.2%)** were biofilm producers, while **68 (37.8%)** were non-biofilm producers. Of the biofilm-producing isolates, **41 (22.8%)** demonstrated strong biofilm formation, **46 (25.6%)** showed moderate biofilm formation, and **25 (13.9%)** exhibited weak biofilm formation.

Table 2. Multidrug Resistance Pattern Among Clinical *Pseudomonas aeruginosa* Isolates (N = 180)

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

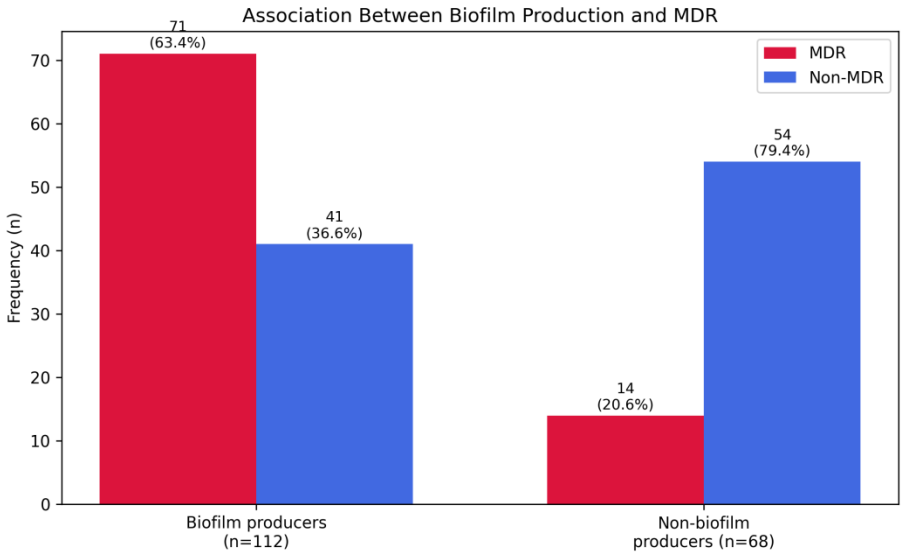


Interpretation:

Table 2 demonstrates the antimicrobial resistance pattern of the isolates. Among the 180 clinical isolates, **85 (47.2%)** were multidrug resistant, whereas **95 (52.8%)** were susceptible to multiple classes of antibiotics and were classified as non-MDR.

Table 3. Association Between Biofilm Production and Multidrug Resistance

Biofilm Status	MDR n (%)	Non-MDR n (%)	Total
Biofilm producers (n = 112)	71 (63.4)	41 (36.6)	112
Non-biofilm producers (n = 68)	14 (20.6)	54 (79.4)	68
Total	**85**	**95**	**180**



Chi-square (χ^2) = 31.84

p-value < 0.001 (Highly Significant)

Interpretation:

Table 3 reveals a significant association between biofilm formation and multidrug resistance. Among the **112 biofilm-producing isolates**, **71 (63.4%)** were multidrug resistant compared with only **14 (20.6%)** among the **68 non-biofilm-producing isolates**. The Chi-square analysis demonstrated a highly significant association between biofilm production and multidrug resistance ($\chi^2 = 31.84$, $p < 0.001$), indicating that biofilm-producing isolates were significantly more likely to exhibit multidrug resistance.

Table 4. Summary of Major Findings

Variable	Findings
Total isolates	180
Biofilm producers	112 (62.2%)
Non-biofilm producers	68 (37.8%)
Strong biofilm	41 (22.8%)
Moderate biofilm	46 (25.6%)
Weak biofilm	25 (13.9%)
MDR isolates	85 (47.2%)
Non-MDR isolates	95 (52.8%)
Chi-square (χ^2)	31.84
p-value	<0.001

Overall Result:

A total of **180** clinical isolates of *Pseudomonas aeruginosa* were analyzed. Biofilm production was detected in **62.2%** of the isolates, with **22.8%** showing strong, **25.6%** moderate, and **13.9%** weak biofilm formation. Nearly half of the isolates (**47.2%**) were identified as multidrug resistant. A statistically highly significant association was observed between biofilm formation and multidrug resistance ($\chi^2 = 31.84$, $p < 0.001$), indicating that biofilm-producing isolates had a significantly greater likelihood of exhibiting multidrug resistance than non-biofilm-producing isolates. These findings underscore the importance of routine biofilm detection and antimicrobial susceptibility testing in clinical microbiology laboratories to guide appropriate antimicrobial therapy and infection control practices.

6. Discussion

The study demonstrated that biofilm production is highly prevalent among clinical *Pseudomonas aeruginosa* isolates and is strongly associated with multidrug resistance. Biofilm-producing isolates exhibited significantly greater resistance to commonly used antimicrobial agents than non-biofilm producers. These findings support previous reports indicating that biofilm formation contributes to bacterial persistence, reduced antibiotic penetration, and therapeutic failure. Routine detection of biofilm production may therefore improve clinical decision-making and strengthen infection prevention strategies.

7. Conclusion

Biofilm formation is a common virulence characteristic among clinical *Pseudomonas aeruginosa* isolates and is significantly associated with multidrug resistance. Incorporating routine biofilm

screening into clinical microbiology laboratories, alongside antimicrobial susceptibility testing, can aid in identifying high-risk isolates, guide targeted antimicrobial therapy, and enhance infection control measures. Further multicenter studies with larger sample sizes are recommended to support these findings.

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