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Biofilm Formation and Its Association With Antimicrobial Resistance in Pseudomonas aeruginosa Clinical Isolates in a Tertiary Care Hospital in Central India

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Abstract

Background

Pseudomonas aeruginosa is a significant opportunistic pathogen in hospital settings, with biofilm formation and antimicrobial resistance being important factors in pathogenicity. Once a biofilm is formed, bacterial persistence ensues, along with decreased antimicrobial susceptibility, making it difficult to eradicate the bacteria. This study was conducted to estimate the rate of biofilm formation in clinical isolates of *P. aeruginosa* and its correlation with multidrug resistance (MDR) and carbapenem resistance.

Methodology

A prospective, hospital-based, cross-sectional study was conducted in the Department of Microbiology, Index Medical College, Hospital & Research Centre, Indore, from January 2023 to December 2025. A total of 171 non-duplicate clinical isolates of *P. aeruginosa* were included. *P. aeruginosa* isolates were subjected to antimicrobial susceptibility testing by the disc diffusion method (Kirby-Bauer method) as per the 2023 Clinical and Laboratory Standards Institute (CLSI) guidelines. The phenotypic detection of biofilm producers was done using the microtiter plate method, and the organisms were graded as weak, moderate, and strong biofilm formers. Data analysis was performed using SPSS Statistics (version 27.0; IBM Corp., Armonk, NY, USA), and the chi-square test was applied to analyze the clinical and laboratory findings. P-values <0.05 were considered statistically significant.

Results

Biofilm was present in 133 (77.8%) isolates, including 31 (18.1%) weak, 55 (32.2%) moderate, and 47 (27.5%) strong producers. In total, 108 (63.2%) isolates were MDR and 93 (54.4%) were carbapenem-resistant. MDR was more frequent among strong biofilm producers than among non-biofilm producers (83.0% vs. 36.8%). Similarly, carbapenem resistance was higher among strong biofilm producers than among non-biofilm producers (80.9% vs. 28.9%). There was a significant association of biofilm with carbapenem-resistant isolates (χ^2= 12.74, p < 0.001). The maximum number of isolates that were positive for biofilm were from respiratory sources (86.1%) and the intensive care unit (ICU) (84.9%).

Conclusions

Biofilm production was common in clinical *P. aeruginosa* and linked to MDR and carbapenem resistance. A larger proportion of clinical isolates were biofilm-positive in ICU and respiratory isolates, emphasizing the need for targeted infection prevention and antimicrobial stewardship and investigation into biofilm-associated resistance.

Categories: Public Health, Infectious Disease
Keywords: antimicrobial resistance, bacterial biofilm, biofilm production, carbapenem-resistant p. aeruginosa, central india region, hospital-aquired infection, intensive care unit, multidrug resistant (mdr), non-fermenting gram-negative bacilli (nfgnb), pseudomonas aeuginosa

Introduction

Pseudomonas aeruginosa is a motile, aerobic, non-fermenting Gram-negative bacillus. *P. aeruginosa* has been isolated from several sources, including hospital sinks, ventilators, humidifiers, and other medical equipment. *P. aeruginosa* is an important pathogen that causes healthcare-associated infections with greater severity of illness in patient populations with a weakened immune system, especially critically ill patients, and also causes community-acquired infections. These infections include ventilator-associated pneumonia, bloodstream infection, urinary tract infection, burn infection, and surgical-site infections [1,2]. Because of this impact on the healthcare system, *P. aeruginosa* is one of the ESKAPE pathogens and the most common

opportunistic pathogen known to acquire resistance to multiple antibiotic classes [3]. Over the past decades, antimicrobial resistance in *P. aeruginosa* has been increasing, as most isolates have acquired multidrug resistance (MDR) and extensive drug resistance (XDR) to different antibiotics [4,5]. Antibiotics and other agents such as carbapenems are used to treat serious infections caused by this organism. In cases unresponsive to antibiotic treatment, the number of carbapenem-resistant *P. aeruginosa* has increased, and their capacity to combat antibiotics has been limited. Resistance mechanisms can include production of carbapenemases such as metallo-β-lactamases (NDM, VIM, AIMP, and other types), overexpression of efflux pumps, and loss of the OprD outer-membrane porin [6,7]. Due to the limited treatment options for these infections, the World Health Organization (WHO) has listed carbapenem-resistant *P. aeruginosa* as a critical priority pathogen for the development of novel antibiotics [8].

Another characteristic of *P. aeruginosa* apart from its intrinsic resistance to antibiotics is its ability to produce biofilms on living and non-living surfaces. Biofilms are communities of bacteria embedded in a self-produced extracellular matrix, which can protect them from the host's immune response and make them less susceptible to antimicrobial agents [9,10]. Therefore, this ability enables the organism to persist on medical devices and hospital surfaces, leading to persistent, chronic, and recurrent infections [11]. Several studies have also identified a potential correlation between biofilm production and antimicrobial resistance in *P. aeruginosa*; however, the strength of this association has differed among various studies in different geographical regions and patient populations [12,13].

The primary objective of this study was to determine the prevalence and degree of biofilm formation among clinical isolates of *P. aeruginosa*. The secondary objectives were to assess the antimicrobial susceptibility patterns of the isolates, particularly MDR and carbapenem resistance; to evaluate the association between the four categories of biofilm formation (negative, weak, moderate, and strong) and MDR and carbapenem resistance; and to describe the distribution of biofilm production according to clinical specimen type and hospital ward.

Materials And Methods

A prospective, hospital-based, cross-sectional study was conducted in the Department of Microbiology, Index Medical College, Hospital & Research Centre (IMCHRC), Indore, from January 2023 to December 2025 after obtaining approval from the Institutional Ethics Committee (reference number: MU/Research/EC/Ph.D/2023/028). The study included non-duplicate, consecutive isolates of *P. aeruginosa* (n = 171) from all patients irrespective of age and sex attending the outpatient and inpatient wards, including intensive care units (ICUs) of the hospital during the study period. Specimens such as respiratory specimens (sputum, endotracheal and tracheal aspirates, endotracheal and tracheostomy tips, and suction specimens), blood, urine, pus, wound swabs, and other sterile body fluids were considered. Patients whose specimens did not contain *P. aeruginosa*, were contaminated, were collected improperly, or were collected without the consent of the patient were excluded from the study.

The minimum sample size was estimated using the following formula [14]: $n = Z^2_{1-\alpha/2} \cdot p \cdot q/d2$, where the expected proportion (p) was taken as 0.20, $q = 1 - p = 0.80$, the confidence level ($1 - \alpha$) was 95% with a corresponding Z-value of 1.96, and the absolute precision (d) was set at 0.06. This gave a sample size of 170.74, which was rounded up to 171 isolates.

The clinical and demographic data were recorded on a predesigned and validated case record form (CRF) which detailed demographic and clinical information, geographical details of the hospital, presence of any comorbidity, clinical specimen type, and details of treatment received by each patient. The CRF was the source document for data collection. All isolates were identified by standard methods, including colony morphology, Gram stain, and catalase, oxidase, motility, urease, and citrate utilization tests, oxidative-fermentative glucose test, nitrate reduction, hemolysis, and growth at 42°C [15]. All media and reagents were checked for sterility and satisfactory performance before use. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar and interpreted as per Clinical and Laboratory Standards Institute (CLSI) M100, 33rd edition (2023) [16] guidelines. *Escherichia coli* ATCC 25922 was used as the control strain. The antimicrobial discs (HiMedia Laboratories, Mumbai, India) used to test the following antibiotics were piperacillin-tazobactam (PIT, 100/10 µg), ceftazidime (CAZ, 30 µg), cefepime (CP, 30 µg), ceftriaxone (CTR, 30 µg), amikacin (AK, 30 µg), gentamicin (GEN, 10 µg), tobramycin (TOB, 10 µg), aztreonam (AT, 30 µg), levofloxacin (LE, 5 µg), imipenem (IMP, 10 µg), and meropenem (MRP, 10 µg). Susceptibility to polymyxin B (PB, 300 units) was estimated using the broth microdilution method and interpreted as per CLSI M100 guidelines [16].

MDR was characterized by non-susceptibility to at least one agent in three or more antimicrobial classes [17]. Carbapenem resistance was characterized by resistance to imipenem and/or meropenem as per interpretation of susceptibility results by CLSI M100, 33rd edition (2023) guidelines [16].

Biofilm formation assay

Biofilm formation was assessed using the microtiter plate method, modified from the procedures described

by Stepanović et al. and O'Toole [18,19]. Sterile brain heart infusion (BHI) broth supplemented with 0.2% glucose, without bacterial inoculum, was used as the negative control to determine background absorbance. *P. aeruginosa* PAO1 (ATCC 15692), a reference strain with established biofilm-forming ability, was used as the positive control. The reference strain and negative-control wells were processed under the same experimental conditions as the clinical isolates. The use of *P. aeruginosa* PAO1 as a positive control for biofilm formation has been described in previous studies [20]. The isolates were grown overnight in BHI broth supplemented with 0.2% glucose (HiMedia Laboratories, Mumbai, India) at 37°C. The overnight cultures were diluted at 1:40 in fresh BHI broth with 0.2% glucose, and 200 µL of each suspension was added to the wells of sterile 96-well polystyrene microtiter plates (Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India), which were then incubated at 37°C for 24 hours.

After incubation, the contents of the wells were discarded, and the wells were gently washed three times with 200 µL of phosphate-buffered saline to remove non-adherent cells. The plates were inverted and air-dried, and the adherent biofilm was stained with 1% crystal violet (HiMedia Laboratories, Mumbai, India) for 15 minutes. The excess stain was removed, and the bound crystal violet was solubilized with an ethanol-acetone mixture (80:20, v/v). The optical density (OD) was read at 620 nm in a microplate reader (Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India, MULTISKAN FC). Based on the OD values, isolates were graded as negative (non-producer), weak, moderate, or strong biofilm producers, using cut-offs of OD <0.275, 0.275-0.55, 0.56-0.825, and ⩾0.826, respectively (Table *1*).

Mean OD value (620 nm)	Biofilm production	Result
<0.275	None	Negative
0.275–0.55	Weak	Positive - weak
0.56–0.825	Moderate	Positive - moderate
≥0.826	Strong	Positive - strong

TABLE 1: Classification of biofilm production based on OD values measured at 620 nm.

OD: optical density

Statistical analysis

Clinical, demographic, and microbiological data were analyzed using SPSS Statistics, version 27.0 (IBM Corp., Armonk, NY, USA). Categorical variables are presented as frequencies and percentages. The association of biofilm production (positive vs. negative) with MDR and carbapenem resistance was assessed using Pearson's chi-square test, with Fisher's exact test used where any expected cell count was below 5. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for each association. For the four biofilm categories (non-producer, weak, moderate, strong), overall differences were assessed by the chi-square test, and a monotonic increase was assessed by the chi-square test for linear trend; each category was compared with non-producers (reference) using Fisher's exact test with Bonferroni correction for three comparisons. Differences in biofilm positivity between ICU and general ward isolates and between specimen types were assessed using the chi-square or Fisher's exact test. Variables with p-values <0.20 in univariable analysis were entered into a multivariable binary logistic regression model to estimate adjusted ORs (aORs) with 95% CIs for the association of biofilm production with MDR and carbapenem resistance, adjusting for specimen type and ICU admission. Model fit was checked using the Hosmer-Lemeshow test. All tests were two-sided, and a p-value <0.05 was considered statistically significant. Because of the cross-sectional design, all results are interpreted as associations and not as evidence of causation.

Results

The study comprised 171 non-duplicate clinical isolates of *P. aeruginosa*, which revealed that the maximum number of *P. aeruginosa* isolates were obtained from the 51-60-year and >60-year age groups (22.2% each), followed by the 41-50-year age group (18.1%). Biofilm formation was observed in 133 (77.8%) isolates. Overall, 38 (22.2%) isolates were non-biofilm producers. Among biofilm producers, 47 (27.5%) isolates had strong biofilm formation, 55 (32.2%) had moderate biofilm formation, and 31 (18.1%) isolates showed weak biofilm formation (Table *2*). The MDR rate and carbapenem resistance rate increased as the biofilm formation strength increased.

Biofilm formation	Isolates, n (%)	MDR, n (%)	Crude OR (95% CI) vs non-producers	Carbapenem-R, n (%)	Crude OR (95% CI) vs non-producers
Strong	47 (27.5%)	39 (83.0%)	8.36 (3.05–22.87)	38 (80.9%)	10.36 (3.78–28.45)
Moderate	55 (32.2%)	38 (69.1%)	3.83 (1.60–9.17)	31 (56.4%)	3.17 (1.31–7.65)
Weak	31 (18.1%)	17 (54.8%)	2.08 (0.79–5.47)	13 (41.9%)	1.77 (0.65–4.82)
Non-producer	38 (22.2%)	14 (36.8%)	1.00 (reference)	11 (28.9%)	1.00 (reference)
Total	171 (100%)	108 (63.2%)	Not applicable	93 (54.4%)	Not applicable

TABLE 2: Distribution of biofilm formation and its association with antimicrobial resistance phenotypes among Pseudomonas aeruginosa isolates (n = 171).

Data are presented as numbers and percentages (n (%)). Percentages for biofilm formation represent the proportion of isolates in each biofilm category relative to the total study population (n = 171). Percentages for MDR and carbapenem resistance are calculated within each biofilm category (row percentages). Crude ORs with 95% CIs were calculated using non-producers as the reference category. The chi-square test for linear trend demonstrated a significant increasing trend in MDR ($\chi^2 = 20.92$, $p < 0.001$) and carbapenem resistance ($\chi^2 = 24.49$, $p < 0.001$) across increasing biofilm grades.

MDR: multidrug resistance; Carbapenem-R: carbapenem resistance; OR: odds ratio; CI: confidence interval

Prevalence of biofilm-producing isolates based on the type of clinical sample and ward is presented in Table 3. The number of biofilm-positive isolates was the highest among respiratory samples (86.1%) and isolates obtained from the ICU (84.9%). Non-biofilm producers were more common among isolates obtained from the ward (38.5%), blood (33.3%), and other specimens (50.0%).

Category	Strong, n (%)	Moderate, n (%)	Weak, n (%)	Negative, n (%)
Respiratory samples (n = 72)	24 (33.3%)	26 (36.1%)	12 (16.7%)	10 (13.9%)
Pus/Wound swab (n = 41)	11 (26.8%)	13 (31.7%)	8 (19.5%)	9 (22.0%)
Urine (n = 28)	6 (21.4%)	9 (32.1%)	6 (21.4%)	7 (25.0%)
Blood (n = 18)	4 (22.2%)	5 (27.8%)	3 (16.7%)	6 (33.3%)
Others (n = 12)	2 (16.7%)	2 (16.7%)	2 (16.7%)	6 (50.0%)
ICU (n = 119)	38 (31.9%)	41 (34.5%)	22 (18.5%)	18 (15.1%)
General wards (n = 52)	9 (17.3%)	14 (26.9%)	9 (17.3%)	20 (38.5%)

TABLE 3: Distribution of biofilm production according to clinical specimen type and hospital ward among Pseudomonas aeruginosa isolates (n = 171).

Data are presented as numbers and percentages (n (%)). Percentages are calculated within each specimen type or ward category. ICU vs. Ward: $\chi^2 = 11.40$, $p < 0.001$; Respiratory vs. other specimens: $\chi^2 = 5.00$, $p = 0.025$.

ICU: intensive care unit

Biofilm production was significantly associated with carbapenem resistance (61.7% (82/133) vs. 28.9% (11/38); crude OR = 3.95, 95% CI: 1.80-8.64; $\chi^2 = 12.74$, df = 1, $p < 0.001$) and with MDR (70.7% (94/133) vs. 36.8% (14/38); crude OR = 4.13, 95% CI: 1.94-8.81; $\chi^2 = 14.54$, df = 1, $p < 0.001$) (Table 4). The proportions of MDR and carbapenem-resistant isolates increased with biofilm strength (MDR: χ^2 for trend = 20.92, $p < 0.001$; carbapenem resistance: χ^2 for trend = 24.49, $p < 0.001$) (Table 5). Compared with non-producers, the odds of carbapenem resistance were higher among moderate (OR = 3.17, 95% CI: 1.31-7.65) and strong producers (OR = 10.36, 95% CI: 3.78-28.45), and the odds of MDR were higher among moderate (OR = 3.83,

95% CI: 1.60-9.17) and strong producers (OR = 8.36, 95% CI: 3.05-22.87) (Tables 6, 7), whereas weak producers did not differ significantly from non-producers. Biofilm positivity was significantly higher among ICU isolates than ward isolates (84.9% vs. 61.5%; OR = 3.51, 95% CI: 1.66-7.43; p < 0.001) and among respiratory than non-respiratory specimens (86.1% vs. 71.7%; OR = 2.45, 95% CI: 1.10-5.43; p = 0.025) (Table 8).

Biofilm production	Carbapenem resistant	Carbapenem sensitive	Crude OR (95% CI)	χ^2	P-value
Positive (strong + moderate + weak)	82	51	3.95 (1.80-8.64)	12.74	<0.001
Strong	38	9			
Moderate	31	24	-	-	
Weak	13	18			
Negative	11	27			

TABLE 4: Association of biofilm production and carbapenem resistance in Pseudomonas aeruginosa (n = 171).

Data are presented as the number of isolates (n). The Pearson chi-square test was used to compare carbapenem resistance between biofilm-positive and biofilm-negative isolates. χ^2 = Pearson chi-square statistic; df = 1.Statistical significance was defined as p < 0.05. Empty cells indicate that the test statistic and p-value are reported for the overall comparison rather than individual rows.

OR: odds ratio; CI: confidence interval

Resistance outcome	Biofilm production grade	Resistant, n/N (%)	Crude OR (95% CI)	Bonferroni-adjusted p-value
MDR	Non-producer	14/38 (36.8%)	1.00 (reference)	Reference
MDR	Weak	17/31 (54.8%)	2.08 (0.79–5.47)	0.46
MDR	Moderate	38/55 (69.1%)	3.83 (1.60–9.17)	0.009
MDR	Strong	39/47 (83.0%)	8.36 (3.05–22.87)	<0.001
Carbapenem-R	Non-producer	11/38 (28.9%)	1.00 (reference)	Reference
Carbapenem-R	Weak	13/31 (41.9%)	1.77 (0.65–4.82)	0.94
Carbapenem-R	Moderate	31/55 (56.4%)	3.17 (1.31–7.65)	0.034
Carbapenem-R	Strong	38/47 (80.9%)	10.36 (3.78–28.45)	<0.001

TABLE 5: Association between biofilm production grade and multidrug resistance and carbapenem resistance among Pseudomonas aeruginosa Isolates (reference = non-producers, n = 38).

Data are presented as the number of resistant isolates divided by the total number of isolates in each biofilm grade, with percentages. Non-producers were considered the reference group for calculating crude ORs and 95% CIs. P-values were calculated using Fisher's exact test for comparisons between each biofilm grade and the reference group. Bonferroni correction was applied for three pairwise comparisons per outcome. A Bonferroni-adjusted p-value <0.05 was considered statistically significant.

MDR: multidrug resistance; Carbapenem-R: carbapenem resistance; OR: odds ratio; CI: confidence interval

Resistance Outcome	Overall χ^2 (df = 3)	Overall P-value	χ^2 for linear trend (df = 1)	Trend p-value	OR per one-grade increase (95% CI)
MDR	21.00	<0.001	20.92	<0.001	2.00 (1.47–2.73)
Carbapenem-R	25.21	<0.001	24.49	<0.001	2.11 (1.55–2.88)

TABLE 6: Overall association and linear trend between biofilm production grade and antimicrobial resistance among Pseudomonas aeruginosa isolates.

Overall associations across the four biofilm production grades were assessed using the Pearson chi-square test (3 degrees of freedom). Linear trends in the proportion of resistant isolates across increasing biofilm grades were assessed using the chi-square test for linear trend (1 degree of freedom). ORs with 95% CIs represent the change in odds of resistance associated with a one-grade increase in biofilm production, with biofilm grades treated as an ordinal variable. A two-sided p-value <0.05 was considered statistically significant.

MDR: multidrug resistance; Carbapenem-R: carbapenem resistance; OR: odds ratio; CI: confidence interval

Outcome	Biofilm-positive, n (%)	Biofilm-negative, n (%)	Crude OR (95% CI)	χ^2 (df = 1)	P-value
Carbapenem resistance	82 (61.7%)	11 (28.9%)	3.95 (1.80–8.64)	12.74	<0.001
MDR	94 (70.7%)	14 (36.8%)	4.13 (1.94–8.81)	14.54	<0.001

TABLE 7: Association between biofilm production and carbapenem resistance and multidrug resistance among Pseudomonas aeruginosa isolates. Biofilm-positive (n = 133) vs biofilm-negative (n = 38).

Data are presented as numbers (percentages). Percentages were calculated within each biofilm group. Crude OR (95% CI) represents the odds of carbapenem resistance or multidrug resistance among biofilm-positive isolates compared with biofilm-negative isolates. Associations were assessed using Pearson's chi-square test (χ^2 (df = 1)). All expected cell counts were greater than 5. Fisher's exact test yielded similar conclusions (carbapenem p = 0.0004; MDR p = 0.0002). A two-sided p-value <0.05 was considered statistically significant.

MDR: multidrug resistance; OR: odds ratio; CI: confidence interval

Comparison (biofilm-positive isolates)	Crude OR (95% CI)	χ^2	P-value
ICU (101/119, 84.9%) vs general ward (32/52, 61.5%)	3.51 (1.66–7.43)	11.40 (df = 1)	<0.001
Respiratory (62/72, 86.1%) vs all other specimens (71/99, 71.7%)	2.45 (1.10–5.43)	5.00 (df = 1)	0.025
Five specimen types, overall	Not applicable	9.66 (df = 4)	0.047

TABLE 8: Association of biofilm positivity with hospital ward and clinical specimen type among Pseudomonas aeruginosa isolates.

Data are presented as the number of biofilm-positive isolates divided by the total number of isolates in each category, with percentages. Crude ORs with 95% CIs compare the odds of biofilm positivity between the specified groups. Associations between hospital ward and biofilm positivity, and between respiratory and non-respiratory specimens, were assessed using Pearson's chi-square test. The overall association between the five specimen types and biofilm positivity was assessed using the Fisher-Freeman-Halton exact test because some expected cell counts were below 5. A two-sided p-value <0.05 was considered statistically significant.

OR: odds ratio; CI: confidence interval; ICU: intensive care unit

Discussion

In the present study, biofilm formation was observed in 77.8% of the clinical isolates of *P. aeruginosa*, of which 27.5% were strong, 32.2% moderate, and 18.1% weak producers. This prevalence is in close agreement with that of Lima et al., who detected biofilm production in 75% of isolates from patients with ventilator-associated pneumonia [20]. A recent study of ICU patients and hospital surfaces from the Western Brazilian

Amazon similarly reported moderate or strong biofilm formation in 77.3% of isolates, although the grading criteria differed from ours [21]. Somewhat higher rates have been reported by Kamali et al., who found biofilm production in 83.75% of clinical isolates [22], and by the meta-analysis of Karballaei Mirzahosseini et al., which reported a pooled rate of 86.5% among clinical isolates from Iran [23].

An important observation in the present study was the progressive increase in antimicrobial resistance across the different biofilm-production categories. The proportion of MDR isolates increased from 36.8% among non-biofilm producers to 83.0% among strong biofilm producers. Similarly, carbapenem resistance increased from 28.9% among non-producers to 80.9% among strong producers. Biofilm production was also associated with MDR (crude OR = 4.13, 95% CI: 1.94-8.81; $p < 0.001$), and both MDR and carbapenem resistance increased across biofilm categories (p for trend <0.001). These are statistical associations in a cross-sectional sample and do not show that biofilm formation causes resistance; the two may coexist because both are more common in isolates from critically ill, device-exposed patients. This pattern suggests that stronger biofilm-forming ability may coexist with a greater burden of antimicrobial resistance among clinical isolates of *P. aeruginosa*. The findings are clinically relevant because infections caused by this organism can be difficult to manage when biofilm-associated persistence occurs alongside established antimicrobial resistance mechanisms.

The relationship between biofilm formation and antimicrobial resistance has also been explored in previous studies. Karballaei Mirzahosseini et al., in their meta-analysis, reported that biofilm formation was more frequent among MDR isolates than among non-MDR isolates. However, a statistically significant association was identified in only half of the studies included in their analysis, indicating that the relationship was not consistent across all study populations [23]. A similar relationship was examined in the meta-analysis by Hadadi-Fishani et al., which evaluated the correlation between biofilm formation and antibiotic resistance in *P. aeruginosa* [24]. These observations suggest that biofilm formation and antimicrobial resistance are associated in many clinical settings, although the strength of the association can vary.

Lima et al. investigated biofilm production among clinical isolates of *P. aeruginosa* obtained from patients with ventilator-associated pneumonia. Using a quantitative method, they detected biofilm production in 75% of the isolates, while 53.3% of the biofilm-producing isolates were MDR. Nevertheless, the authors concluded that biofilm production was independent of the antimicrobial susceptibility profile in their study population [19]. Their findings differ from the progressive increase in MDR observed across biofilm categories in the present study. This difference may reflect variations in sample size, study population, laboratory methods, and resistance patterns; in particular, Lima et al. examined only 20 isolates, which limits the extent to which their findings can be generalized [20]. Taken together, these studies suggest that although biofilm formation may be associated with antimicrobial resistance, the relationship should be evaluated within the context of the individual study population rather than assumed to be universal.

The association between biofilm formation and carbapenem resistance was particularly noteworthy in the present study. A statistically significant association was observed between these variables ($p < 0.001$), indicating that the distribution of carbapenem resistance differed significantly according to biofilm-production status. This finding is important because carbapenems are valuable therapeutic agents for serious infections caused by resistant Gram-negative bacteria, including *P. aeruginosa*. The higher carbapenem resistance rate among strong biofilm producers raises concern about the coexistence of resistance mechanisms and biofilm-associated persistence, both of which may complicate infection management.

The findings also have potential clinical relevance for infections involving respiratory specimens and patients admitted to ICUs. *P. aeruginosa* can survive in moist hospital environments and colonize invasive medical devices, including endotracheal tubes and respiratory equipment. Biofilm formation on these surfaces may contribute to persistent colonization and can be relevant to the development and management of healthcare-associated infections. In the present study, biofilm positivity was highest among respiratory isolates (86.1%) and ICU isolates (84.9%), compared with 61.5% among isolates from general wards. Lima et al. similarly demonstrated the relevance of biofilm production in isolates from patients with ventilator-associated pneumonia [20].

The present findings also contribute to the available evidence on biofilm formation and antimicrobial resistance in Indian healthcare settings. Saha et al. investigated biofilm production and its relationship with antimicrobial resistance among clinical isolates of *P. aeruginosa* in a tertiary-care hospital in northeastern India [25]. Their study provides a relevant regional comparison because both investigations examine clinical isolates in hospital settings. Nevertheless, direct comparisons of prevalence and resistance patterns should account for differences in study design, patient characteristics, specimen distribution, and laboratory methods. The findings from the present study in central India add to this evidence by demonstrating a high prevalence of biofilm formation and a progressive increase in MDR and carbapenem resistance across the biofilm-production categories.

These observations highlight the importance of infection prevention and antimicrobial stewardship in reducing the burden of infections caused by resistant *P. aeruginosa*. Appropriate hand hygiene,

environmental cleaning, maintenance of medical equipment, and adherence to infection-control protocols remain essential measures in healthcare settings. Particular attention should be paid to invasive respiratory devices and other surfaces that may support biofilm development. In addition, timely detection of carbapenem-resistant isolates can help guide treatment decisions and support measures to prevent the spread of resistant organisms. Further research is required to determine whether routine assessment of biofilm formation provides additional clinical information beyond conventional antimicrobial susceptibility testing.

This study has certain limitations. First, it was conducted at a single tertiary care hospital, which may limit the generalizability of the findings. Second, the cross-sectional design limits the ability to establish temporal or causal relationships between biofilm formation and antimicrobial resistance. Third, biofilm production was assessed using a phenotypic microtiter plate method without molecular characterization of biofilm-associated genes or antimicrobial resistance mechanisms. Furthermore, the study did not evaluate patient-level clinical outcomes, such as treatment response, length of hospital stay, or mortality. The possibility of selection bias and confounding by clinical factors, particularly ICU admission and specimen type, should also be considered when interpreting the findings. Finally, the study did not establish whether all respiratory isolates represented active infection rather than colonization. Despite these limitations, the study provides useful information regarding biofilm formation and antimicrobial resistance among clinical *P. aeruginosa* isolates in central India.

Conclusions

The present study demonstrated a high prevalence of biofilm formation among clinical isolates of *P. aeruginosa*, with 77.8% of isolates exhibiting biofilm-producing ability. A significant association was observed between biofilm positivity and antimicrobial resistance, particularly multidrug and carbapenem resistance. Notably, the proportions of MDR and carbapenem-resistant isolates increased progressively with increasing biofilm production. Strong biofilm producers showed substantially higher odds of both resistance phenotypes compared with non-producers. Biofilm positivity was also more frequent among isolates obtained from ICU patients and respiratory specimens. These findings suggest that biofilm-forming ability and antimicrobial resistance frequently coexist among clinical *P. aeruginosa* isolates. This association may reflect the coexistence of distinct bacterial phenotypes; however, the underlying molecular mechanisms and any direct relationship between biofilm formation and antimicrobial resistance could not be established in the present study. The findings highlight the importance of antimicrobial stewardship and infection prevention and control measures, particularly in intensive care settings. Further multicenter studies incorporating molecular characterization and patient-level clinical outcomes are warranted to clarify the relationship between biofilm formation, antimicrobial resistance, and their clinical significance.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Malwanchal University Ethics Committee issued approval MU/Research/EC/Ph.D/2023/028. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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