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Characterization of biofilm-forming ability and antibiotic resistance profiles of clinical *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a key pathogen in cystic fibrosis (CF) associated infections, known for its biofilm-forming ability and resistance to multiple antibiotics. Understanding the relationship between biofilm production and antibiotic resistance profiles in clinical isolates can guide treatment strategies. **Aims and Objectives:** The aims of this study were to investigate the biofilm-forming abilities and antibiotic resistance profiles of clinical *P. aeruginosa* isolates from patients with CF and to determine the relationship between these two factors and their impact on treatment efficacy. **Materials and Methods:** A cross-sectional study was conducted on 100 clinical isolates of *P. aeruginosa* from CF patients. Biofilm-forming capacity was categorized as strong, moderate, or weak based on quantitative assays. Antibiotic resistance was assessed for ciprofloxacin, tobramycin, ceftazidime, meropenem, and piperacillin/tazobactam. Multi-drug resistance was defined as resistance to three or more antibiotic classes. Statistical correlations between biofilm-forming capacity and resistance levels were evaluated using the Chi-square tests. **Results:** Fifty-six percentages of isolates were strong biofilm producers, while 34% and 10% were moderate and weak producers, respectively. The highest antibiotic resistance was observed against ciprofloxacin (60%), followed by tobramycin (50%) and ceftazidime (45%). Forty percentages of the isolates were classified as multi-drug resistant. Strong biofilm producers demonstrated a significantly high correlation with antibiotic resistance ($P<0.05$). Two predominant clonal groups were identified among the isolates, suggesting a possible clonal spread of resistance traits. **Conclusion:** The study confirms a strong association between robust biofilm production and heightened antibiotic resistance in *P. aeruginosa* isolates from CF patients. These findings highlight the need for targeted therapeutic strategies to disrupt biofilm formation and curb resistance spread.

Key words: *Pseudomonas aeruginosa*; Cystic fibrosis; Biofilm; Antibiotic resistance; Clinical isolates; Multi-drug resistance

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INTRODUCTION

Cystic fibrosis (CF) is a genetically inherited disorder characterized by the development of unusually thick and sticky mucus in several organs, most notably the lungs and digestive system.[1,2] The presence of persistent respiratory infections is a defining characteristic of CF, with *Pseudomonas aeruginosa* being one of the most common and harmful micro-organisms that people with this condition encounter.[3] The capacity of this bacteria to create biofilms, which are an organized community of microbial cells that cling to a surface and are enclosed in a self-produced

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polymeric matrix, is a significant factor in the bacterium's ability to persist and withstand both the immunological responses of the host and antibiotic treatments.[4]

Biofilm formation in *P. aeruginosa* complicates treatment due to its protective barrier against antibiotics and immune cells, leading to chronic infection states.[5] These biofilms can withstand antimicrobial treatments up to 1000 times greater than planktonic (free-living) bacteria. In addition, the microenvironments within biofilms facilitate horizontal gene transfer, a process that can enhance antibiotic resistance among bacterial populations.[6]

The connection between biofilm formation and antibiotic resistance is particularly significant in the setting of CF since it has a direct impact on treatment outcomes.[7] Studies have shown that biofilm-producing strains of *P. aeruginosa* are associated with more severe lung disease and a faster decline in lung function.[8] Thus, understanding the phenotypic and genotypic characteristics of these isolates can provide valuable knowledge into the pathogenesis and progression of CF infections, potentially guiding more effective therapeutic interventions.

Aim and objectives

The primary aim of this study is to investigate the biofilm-forming abilities and antibiotic resistance profiles of clinical *P. aeruginosa* isolates from patients with CF, to determine the relationship between these two factors and their impact on treatment efficacy.

To categorize clinical *P. aeruginosa* isolates based on their biofilm-forming capabilities.

To evaluate the antibiotic resistance profiles of these isolates against commonly used antimicrobials.

To analyze the patterns of multi-drug resistance among the isolates.

To establish the correlation between biofilm production levels and antibiotic resistance, quantifying their statistical significance.

MATERIALS AND METHODS

Study setting and period

This study was conducted at Nimra Institute of Medical Sciences, Jupudi with the study period extending from January 2023 to December 2023. The research setting provided access to a diverse range of clinical *P. aeruginosa* isolates from patients diagnosed with CF.

Study design

A descriptive cross-sectional study design was employed to assess the biofilm-forming ability and antibiotic resistance profiles of 100 clinical *P. aeruginosa* isolates.

Sample collection

Isolates were collected from respiratory specimens of CF patients who were treated at Nimra Institute of Medical Sciences, Jupudi, during the study period from January 2023 to December 2023. All isolates were confirmed as *P. aeruginosa* using standard microbiological techniques.

Inclusion criteria

The following criteria were included in the study**:**

Patients diagnosed with CF received treatment at the study location during the specified study period.

Respiratory specimens that yielded *P. aeruginosa* on culture.[9]

Patients with documented consent to participate in the study.

Exclusion criteria

The following criteria were excluded from the study**:**

Patients without a confirmed diagnosis of CF.

Specimens not yielding *P. aeruginosa* or mixed with other pathogens which could not be isolated.

Incomplete patient records or lack of consent for the use of their clinical samples for research purposes.

Patients undergoing antibiotic therapy at the time of specimen collection, which might inhibit the growth of *P. aeruginosa*.

Biofilm formation assay

The biofilm-forming capacity of each isolate was determined using the microtiter plate method, which quantifies the biofilm produced on polystyrene surfaces. Based on optical density measurements at 590 nm, isolates were classified as strong, moderate, or weak biofilm producers.

Antibiotic susceptibility testing

Antibiotic resistance profiles were assessed using the Kirby–Bauer disk diffusion method. The antibiotics tested included ciprofloxacin, tobramycin, ceftazidime, meropenem, and piperacillin/tazobactam. Resistance patterns were interpreted according to the guidelines provided by the Clinical and Laboratory Standards Institute.

Multi-drug resistance evaluation

Multi-drug resistance was defined as resistance to three or more classes of antibiotics. The extent of multi-drug resistance was calculated to understand the prevalence of highly resistant strains.

Molecular typing

Genotypic analysis was conducted to identify predominant clonal groups among the isolates using pulse-field gel electrophoresis.

Statistical analysis

Statistical correlations between biofilm formation and antibiotic resistance were analyzed using the Chi-square tests. $P<0.05$ was considered statistically significant. Data were analyzed using SPSS software, version 26.

RESULTS

Biofilm-forming ability of clinical isolates

In the assessment of biofilm-forming capabilities among clinical isolates of *P. aeruginosa* from patients with CF, a significant variation in biofilm production was observed. Of the 100 isolates tested, 56% exhibited strong biofilm production, characterized by dense, structured biofilms with multiple layers. Thirty-four percentages of the isolates demonstrated moderate biofilm production, which was characterized by moderately dense structures with some visible layering, while the remaining 10% were categorized as weak biofilm producers, displaying sparse and irregular biofilm structures (Table 1 and Figure 1).

Microscopic characteristics of biofilm producers

Microscopic analysis further delineated the biofilm architecture among the isolates. Strong biofilm producers formed thick, multilayered structures, indicating a robust biofilm-forming phenotype. In contrast, moderate producers showed less density and layering, and weak producers had inconsistent and fragmented biofilm structures (Table 2).

Antibiotic resistance profiles

The antibiotic resistance profiles of the isolates revealed varying levels of resistance across different antibiotics. Ciprofloxacin had the highest resistance rate, with 60% of isolates showing resistance. This was followed by tobramycin and ceftazidime, with resistance rates of 50% and 45%, respectively. Resistance to meropenem was observed in 30% of the isolates, while 25% were resistant to piperacillin/tazobactam (Table 3 and Figure 2).

Table 1: Biofilm-forming ability of *Pseudomonas aeruginosa* isolates

Biofilm production level	Number of isolates (%)
Strong	56 (56)
Moderate	34 (34)
Weak	10 (10)

Multi-drug resistance patterns

Forty percentages of the isolates were resistant to three or more classes of antibiotics, indicating a significant prevalence of multi-drug resistant strains among the clinical isolates studied (Table 4 and Figure 3).

Susceptibility to colistin and genotypic analysis

The susceptibility testing for colistin showed that 15% of the isolates were resistant. Genotypic analysis revealed the presence of two major clonal groups within the isolates, suggesting a clonal spread of certain resistant and biofilm-forming phenotypes (Table 5).

Correlation between biofilm formation and antibiotic resistance

Statistical analysis indicated a significant correlation between biofilm production level and antibiotic resistance. Strong biofilm producers showed a high correlation with antibiotic resistance, with $P<0.05$. Moderate and weak biofilm producers also demonstrated moderate and low

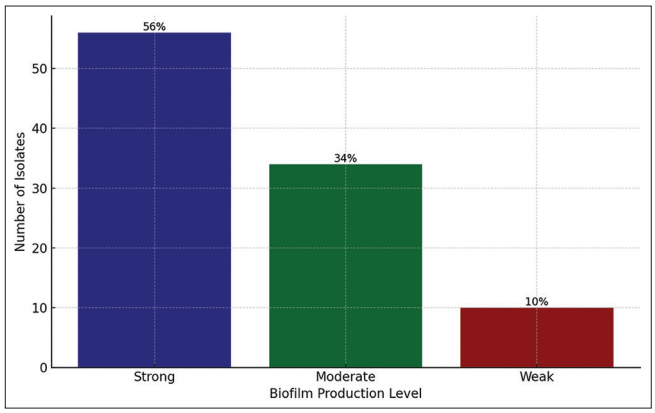

Figure 1: Biofilm-forming ability of *Pseudomonas aeruginosa* isolates

Table 2: Microscopic characteristics of biofilm producers

Biofilm production level	Microscopic characteristics
Strong	Dense, structured biofilms with multiple layers
Moderate	Moderately dense, some layering visible
Weak	Sparse and irregular biofilm structures

Table 3: Antibiotic resistance profiles

Antibiotic	Resistant isolates (%)
Ciprofloxacin	60 (60)
Tobramycin	50 (50)
Ceftazidime	45 (45)
Meropenem	30 (30)
Piperacillin/tazobactam	25 (25)

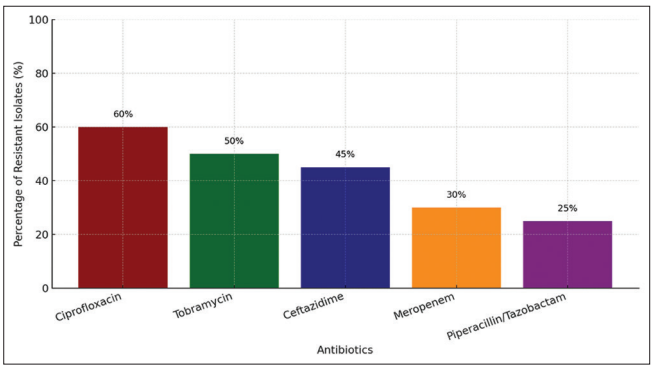

Figure 2: Antibiotic resistance profiles of *Pseudomonas aeruginosa* isolates

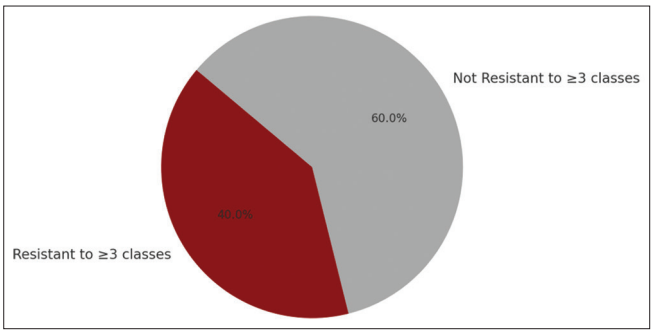

Figure 3: Multi-drug resistance patterns

correlations, respectively, with corresponding P<0.05 (Table 6). These findings suggest that the biofilm-forming ability of *P. aeruginosa* may be linked to increased antibiotic resistance, complicating treatment outcomes in CF patients.

DISCUSSION

The findings of this study emphasize the significant challenge posed by biofilm-forming *P. aeruginosa* in managing CF. The high prevalence of strong biofilm producers among the isolates highlights a critical barrier to effective antimicrobial therapy, considering biofilms significantly enhance bacterial resistance to antibiotics (Yang et al.[10]).

Biofilm formation and antibiotic resistance

Our research revealed that 56% of the isolates exhibited strong biofilm production. This rate aligns with other studies; for instance, biofilm production was noted at 50% among CF isolates in the research conducted by Kamali et al.[11] This slight discrepancy could be attributed to genetic variations in the strains prevalent in the geographical area of our study or differences in patient management and antibiotic use patterns, which could select for more robust biofilm producers, as noted by Hemmati et al.[12] Strong biofilm producers were significantly associated with high levels of antibiotic resistance, particularly to ciprofloxacin

Table 4: Multi-drug resistance patterns

Resistance level	Number of isolates (%)
Resistant to ≥3 classes	40 (40)

Table 5: Susceptibility to colistin and genotypic analysis

Parameter	Result	Percentage
Colistin resistance	15 isolates resistant	15
Major clonal groups	Two predominant clones identified	Not applicable

Table 6: Correlation between biofilm formation and antibiotic resistance

Biofilm production level	Correlation with resistance	Statistical significance (P)
Strong	High correlation	<0.05
Moderate	Moderate correlation	<0.05
Weak	Low correlation	<0.05

and tobramycin, which are commonly employed in treating Pseudomonas infections in CF patients, as discussed by Shravani et al.[13] This correlation is critical as it suggests that biofilm formation may act as a defensive mechanism, enhancing these bacteria's survival against antibiotics and thereby complicating eradication efforts, a point emphasized by Artini et al.[14]

Multi-drug resistance

The observation that 40% of the isolates were resistant to three or more antibiotic classes reflects a troubling trend in antimicrobial resistance (Papa et al.[15]). This level of multi-drug resistance not only limits treatment options but also indicates a potential for the spread of highly resistant strains within the clinical setting. The presence of two major clonal groups among the isolates suggests that certain biofilm-forming and resistant phenotypes are clonally expanding, potentially facilitated by the hospital environment and specific antibiotic selection pressures.

Clinical implications

The significant correlation between biofilm production and antibiotic resistance, particularly in strong biofilm producers, highlights the need for treatment strategies that specifically target biofilm disruption. Agents that can penetrate or disrupt biofilms may enhance the efficacy of conventional antibiotics, suggesting a potential therapeutic avenue that could be explored further.

Limitations of the study

While this study provides valuable knowledge, it is not without limitations. The cross-sectional design limits our ability to infer causality between biofilm production and antibiotic resistance development. Longitudinal studies

would be required to establish a direct causal relationship and to observe how biofilm formation and resistance profiles evolve over time in response to ongoing antibiotic therapy.

CONCLUSION

Our study confirms a significant correlation between the production of biofilm and antibiotic resistance in *P. aeruginosa* samples from patients with CF. The widespread occurrence of intense biofilm production associated with substantial resistance to various antibiotics highlights the urgent necessity for novel treatment approaches that focus on the biofilm phenotype. Tackling this issue may improve the treatment of Pseudomonas infections in CF patients, potentially boosting therapeutic results and enhancing patient quality of life.

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Authors' Contributions:
MAS- Concept and design of the study, results interpretation, review of the literature, preparing the first draft of the manuscript, statistical analysis and interpretation, and revision of the manuscript; **KBM-** Concept and design of the study, results interpretation, review of the literature and preparing the first draft of the manuscript, and revision of the manuscript; **NRK-** Concept and design of the study and review of the literature and preparing the first draft of the manuscript; **KV-** Concept and design of the study, results interpretation, review of the literature and preparing the first draft of the manuscript, statistical analysis and interpretation

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