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Detection of Biofilm Formation in Gram-Positive and Gram-Negative Bacteria from Clinical Samples and their Antibiogram in a Tertiary Care Hospital, Lucknow

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Abstract: ***Background: Biofilm-forming bacteria are recognized as a major contributor to persistent infections, particularly in hospital and device-associated settings. The structural and functional complexity of biofilms provides bacteria with enhanced protection against antimicrobial agents, leading to chronic infections, prolonged hospital stays, and frequent treatment failures. Objectives: This study aimed to detect biofilm formation among Gram-positive and Gram-negative clinical isolates and to assess the correlation between biofilm production and antimicrobial resistance patterns. Methods: A cross-sectional study was carried out in the Microbiology Department of a tertiary care hospital in Lucknow. A total of 150 clinical isolates were obtained from various specimens, including blood, urine, pus, and respiratory samples. Biofilm formation was evaluated using the Congo Red Agar (CRA) method, and antimicrobial susceptibility testing was performed following CLSI guidelines using the Kirby-Bauer disc diffusion method. Results: Out of 150 isolates, 18% were identified as strong biofilm producers, 17.33% as weak producers, and 64.67% as non-biofilm producers. Among the isolates, Escherichia coli and methicillin-resistant coagulase-negative staphylococci (MRCoNS) showed the highest biofilm-producing ability. A significant association was observed between biofilm formation and resistance to ciprofloxacin, cefoxitin, and ceftriaxone ($p < 0.05$). Conclusion: The study highlights the growing prevalence of biofilm-forming, multidrug-resistant pathogens in clinical settings. Routine screening for biofilm formation should be implemented to guide antibiotic therapy and improve clinical outcomes.***

Keywords: Biofilm, Antibiotic resistance, Congo Red Agar, Gram-positive bacteria, Gram-negative bacteria, Multidrug resistance

1. Introduction

Biofilms are structured microbial communities enclosed within a self-produced extracellular polymeric matrix, which facilitates adherence to biotic and abiotic surfaces. These sessile communities exhibit markedly increased tolerance to antimicrobial agents and host immune responses compared to their planktonic counterparts. Biofilm formation is now recognized as a major factor contributing to persistent and recurrent infections, especially in association with medical devices such as catheters, prosthetic implants, and ventilators.

Both Gram-positive and Gram-negative bacteria possess the ability to form biofilms, which act as a protective niche against antibiotic penetration and immune clearance. As a result, biofilm-associated infections are often difficult to eradicate and can lead to prolonged hospital stays, higher healthcare costs, and increased morbidity. Notably, pathogens like *Escherichia coli*, *Staphylococcus aureus*, and coagulase-negative staphylococci are frequently implicated in biofilm-related infections.

The emergence and spread of multidrug-resistant (MDR) organisms have further intensified the challenge of managing biofilm-associated infections. Biofilm production not only facilitates horizontal gene transfer, promoting the spread of resistance genes, but also diminishes the efficacy of conventional antibiotic therapies.

Given these clinical implications, routine detection of biofilm formation in clinical isolates is crucial for devising effective infection control strategies and optimizing antimicrobial therapy. This study was undertaken to evaluate the biofilm-forming ability of Gram-positive and Gram-negative bacteria isolated from clinical specimens and to analyze the relationship between biofilm production and antibiotic resistance profiles.

2. Materials and Methods

Study Design and Setting

This cross-sectional study was conducted over a period of six months in the Department of Medical Microbiology, Integral Institute of Medical Sciences and Research (IIMSR), Lucknow, a tertiary care hospital catering to a wide patient population.

Sample Collection

A total of 150 non-duplicate clinical isolates were obtained from various clinical specimens, including urine, pus, blood, sputum, and wound swabs. All specimens were collected aseptically following standard microbiological protocols and processed promptly to avoid contamination and overgrowth of commensals.

Isolation and Identification of Bacterial Isolates

Bacterial pathogens were isolated and identified based on conventional microbiological techniques, including colony morphology, Gram staining, and standard biochemical tests as per Clinical and Laboratory Standards Institute (CLSI) guidelines (2023).

Detection of Biofilm Formation

Biofilm production was evaluated using the Congo Red Agar (CRA) method. The CRA medium was prepared by supplementing Brain Heart Infusion agar with 0.8 g/L Congo

Red dye and 36 g/L saccharose. Bacterial isolates were inoculated and incubated at 37°C for 24–48 hours. Biofilm-forming ability was interpreted based on colony morphology:

- Strong biofilm producers – black, dry crystalline colonies
- Weak biofilm producers – dark red colonies
- Non-producers – pink or red smooth colonies

Antibiotic Susceptibility Testing (AST)

Antimicrobial susceptibility of the isolates was determined by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following CLSI (2023) guidelines. The antibiotic panel included:

- For Gram-negative bacteria: ciprofloxacin (5 µg), amikacin (30 µg), ceftriaxone (30 µg), imipenem (10 µg), and piperacillin-tazobactam (100/10 µg).
- For Gram-positive bacteria: cefoxitin (30 µg), teicoplanin (30 µg), linezolid (30 µg), erythromycin (15 µg), and vancomycin (30 µg).

Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antibiotic classes.

Statistical Analysis

All data were analyzed using SPSS software version 23.0 (IBM, USA). The Chi-square test was applied to determine the association between biofilm formation and multidrug resistance. A p-value of <0.05 was considered statistically significant.

3. Result

Distribution of Clinical Isolates

A total of 150 clinical bacterial isolates were obtained from various specimens, including urine, blood, pus, sputum, and wound swabs. *Escherichia coli* was the most common isolate, accounting for 37.3% (n = 56), followed by *Klebsiella pneumoniae* (18.0%, n = 27), *Pseudomonas aeruginosa* (12.0%, n = 18), *Staphylococcus aureus* (12.0%, n = 18), methicillin-resistant coagulase-negative staphylococci (MRCoNS) (10.0%, n = 15), and other bacterial species (10.7%, n = 16) (Table 1).

Table 1: Distribution of Clinical Isolates (n = 150)

Category	Number of Isolates	Percentage (%)
E. coli	56	37.3%
K. pneumoniae	27	18.0%
P. aeruginosa	18	12.0%
S. aureus	18	12.0%
MRCoNS	15	10.0%
Others	16	10.7%

Biofilm Formation Among Isolates

Out of the 150 isolates tested, 27 (18.0%) were identified as strong biofilm producers, 26 (17.3%) as weak biofilm producers, and the remaining 97 (64.7%) were classified as non-producers. The majority of strong biofilm producers were *E. coli* and MRCoNS (Table 2).

Table 2: Biofilm Formation by Clinical Isolates

Category	Number of Isolates	Percentage (%)
Strong biofilm producers	27	18.0%
Weak biofilm producers	26	17.3%
Non-biofilm producers	97	64.7%

Antibiotic Resistance Patterns

Biofilm-producing isolates exhibited significantly higher resistance rates compared to non-producers. Resistance to ciprofloxacin, ceftriaxone, and cefoxitin was particularly high among biofilm producers (82.1%, 75.6%, and 88.9%, respectively). In contrast, teicoplanin and linezolid remained effective against most Gram-positive organisms. The comparative resistance patterns of biofilm producers versus non-producers are summarized in Table 3.

Table 3: Antibiotic Resistance in Biofilm Producers vs. Non-Producers

Antibiotic	Resistance in Biofilm Producers (%)	Resistance in Non-Producers (%)
Ciprofloxacin	82.10%	46.30%
Ceftriaxone	75.60%	41.20%
Cefoxitin (MRCoNS)	88.90%	33.30%

Statistical Association

Biofilm production was found to be significantly associated with multidrug resistance (MDR) (Chi-square = 11.6, p = 0.001). The odds of MDR were 3.8 times higher among biofilm producers compared to non-producers.

4. Discussion

Biofilm-forming bacteria play a crucial role in the persistence, recurrence, and chronicity of infections, particularly in healthcare settings. In the present study, 35.33% of isolates were identified as biofilm producers, which aligns with findings reported in other regional studies where the prevalence ranged between 30%–40% among clinical isolates. The predominance of *E. coli* and MRCoNS as biofilm producers in our study is consistent with previous research that identifies these organisms as leading causes of device-associated and hospital-acquired infections.

A significant association between biofilm formation and multidrug resistance (MDR) was observed, with biofilm producers exhibiting notably higher resistance to ciprofloxacin, ceftriaxone, and cefoxitin. Similar trends have been documented in earlier studies, which highlight that the protective extracellular matrix of biofilms hinders antibiotic penetration and facilitates horizontal gene transfer, thereby enhancing antimicrobial resistance.

The Congo Red Agar (CRA) method used in this study proved to be simple, reliable, and cost-effective for the phenotypic detection of biofilms. Although CRA does not provide quantitative assessment, its practicality makes it a valuable screening tool, especially in resource-limited laboratories where advanced techniques such as microtiter plate assays or confocal microscopy may not be feasible.

These findings underscore the importance of incorporating biofilm detection into routine diagnostic workflows, particularly for isolates from patients with recurrent or device-associated infections. Early identification of biofilm-producing strains, combined with targeted antibiotic therapy, can play a critical role in improving treatment outcomes and minimizing the spread of resistant pathogens.

5. Conclusion

This study highlights a considerable prevalence of biofilm-forming and multidrug-resistant clinical isolates, with *E. coli* and MRCoNS emerging as the dominant biofilm producers. The significant correlation between biofilm production and antibiotic resistance emphasizes the necessity of routine biofilm screening alongside conventional antibiograms in clinical microbiology laboratories. Implementing these measures, along with prudent antibiotic stewardship, is vital for improving patient outcomes, reducing treatment failures, and curbing the rise of antimicrobial resistance in hospital settings.

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[1] The author utilized ChatGPT to assist with language refinement and vocabulary enhancement during the preparation of this manuscript. All content generated was thoroughly reviewed and edited by the author, who takes full responsibility.
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