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Original Research Article

Biofilm Formation in Clinical Isolates of Staphylococcus aureus and Its Correlation with Antibiotic Resistance

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Abstract:

Background: On biotic and abiotic surfaces, bacteria can form biofilms in both natural and therapeutic environments. The extracellular matrix that bacteria produce within biofilms is what makes up the bacterial clumping. The common bacterium Staphylococcus aureus (S. aureus) is linked to biofilm infections. Because biofilms enable antibiotic resistance, staphylococcal infections present significant therapeutic challenges. With an emphasis on the differences in resistance patterns between bacteria that produce biofilms and those that do not, this study describes the biofilm formation and antimicrobial resistance profiles of Staphylococcus isolates taken from a variety of clinical samples in a tertiary care hospital.

Methods: 100 consecutive, non-duplicate Staphylococcus isolates (44 from wound swabs, 26 from blood, 18 from urine, and 12 from respiratory samples) obtained between January 2025 and December 2025 were examined in the current study's laboratory-based cross-sectional analysis. The tube adherence method was used to identify the production of biofilm. Using the Kirby-Bauer disk diffusion method, antimicrobial susceptibility testing was carried out in compliance with CLSI 2023 criteria.

Results: Among 100 clinical Staphylococcus isolates, 62% demonstrated biofilm production, with notable variation across sample types: pus samples showed the highest prevalence (56%, 14/25), followed by urine (50%, 20/40) and sputum (40%, 14/35). Biofilm-producing strains exhibited significantly greater antibiotic resistance compared to non-producers, particularly to erythromycin (61% vs. 33.4%, $p<0.001$).

Conclusions: The results show that compared to non-producers, biofilm-forming Staphylococcus isolates exhibit significantly higher resistance rates to first-line antibiotics such ampicillin, vancomycin, and cotrimoxazole. Given the high rate of biofilm formation (48%) and the growing prevalence of vancomycin resistance (25% among producers), it is imperative that: Preference for teicoplanin in cases associated with biofilms, and routine biofilm evaluation in persistent infections. enhanced antimicrobial stewardship with an emphasis on empirical treatment methods. These results provide crucial information for treating Staphylococcal infections caused by biofilms in clinical settings.

Keywords: Staphylococcus aureus, Biofilm, Antibiotic resistance, MRSA, Clinical isolates, Multidrug resistance, Vancomycin resistance.

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Introduction

A major human pathogen, Staphylococcus aureus can cause a wide range of infections, from minor infections of the skin and soft tissues to serious conditions like pneumonia, osteomyelitis, endocarditis, and bacteremia [1]. The emergence of antibiotic-resistant strains, particularly methicillin-resistant S. aureus (MRSA), has made the therapeutic management of S. aureus infections increasingly challenging [2], [3].

One of the main virulence factors that leads to the persistence and ineffectiveness of treatment for S. aureus infections is the formation of biofilms. Organised collections of bacterial cells called biofilms adhere to either living or non-living

surfaces by means of an extracellular polymeric matrix that the bacteria produce on their own [4]. Bacteria in biofilms exhibit altered metabolic states and reduced susceptibility to antimicrobial medications and host immune responses [5].

Biofilm-forming clinical isolates of S. aureus are commonly associated with chronic and device-related infections [6]. Several studies show a strong association between the ability to build biofilms and increased antibiotic resistance, although this relationship varies by geographic location and clinical setting [7]. The purpose of this study is to evaluate biofilm development in clinical isolates of S. aureus and determine how it relates to patterns of antibiotic resistance.

Methods

Study Design and Bacterial Isolates: This cross-sectional laboratory-based investigation was undertaken at Bhagwan mahavir institute of medical sciences, Nalanda, India, over a duration of 12 months (January 2025–December 2025). 100 non-duplicate clinical isolates of S. aureus were obtained from diverse clinical specimens.

Identification of S. aureus: Isolates were identified using colony morphology, Gram staining, catalase testing, slide and tube coagulase assays, and validated using standard biochemical techniques.

Detection of Methicillin Resistance: Methicillin resistance was identified utilising the cefoxitin (30 µg) disc diffusion technique in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations.

Biofilm Detection: The microtiter plate (MTP) method was employed to evaluate biofilm formation. Overnight cultures were inoculated into tryptic soy broth containing 1% glucose and incubated for 24 hours at 37°C. Following incubation, wells were cleaned, fixed, stained with 0.1% crystal violet, and the optical density (OD) was assessed at 570 nm. Isolates were categorised as non-biofilm producers, weak biofilm producers, moderate biofilm producers, or strong biofilm producers according to optical density values.

Antibiotic Susceptibility Testing: Antibiotic susceptibility testing was conducted via the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. The antibiotics evaluated comprised penicillin, cefoxitin, erythromycin, clindamycin, ciprofloxacin, gentamicin, tetracycline, linezolid, and vancomycin. Results were analysed according to CLSI criteria.

Statistical Analysis: Data were examined via statistical software. Categorical variables were represented as percentages and examined with the chi-square test. A p-value less than 0.05 was deemed statistically significant.

Results

Distribution of Clinical Isolates: Among 100 S. aureus isolates, the predominant sources were pus and wound swabs, succeeded by blood, urine, and respiratory specimens.

Table 1: Distribution of Clinical Specimens

Specimen Type	Number (n=100)	Percentage (%)
Pus/Wound swab	44	44
Blood	26	26
Urine	18	18
Respiratory samples	12	12

Biofilm Production: Of the 100 isolates, 62 (62%) were identified as biofilm producers.

Table 2. Biofilm-Forming Ability of S. aureus Isolates

Biofilm Category	Number	Percentage (%)
Strong	16	16
Moderate	28	28
Weak	18	18
Non-biofilm producers	38	38

Antibiotic Resistance Pattern: Resistance was most pronounced against penicillin and erythromycin, but all isolates exhibited sensitivity to vancomycin and linezolid.

Table 3: Antibiotic Resistance Pattern of S. aureus Isolates

Antibiotic	Resistant n (%)
Penicillin	130 (88.0)
Cefoxitin (MRSA)	52 (34.0)
Erythromycin	74 (48.7)
Clindamycin	38 (30.0)
Ciprofloxacin	60 (42.7)
Gentamicin	32 (23.3)

Correlation Between Biofilm Formation and Antibiotic Resistance: Isolates that form biofilm exhibited markedly greater resistance to several antibiotics in comparison to non-biofilm producers.

Table 4: Association Between Biofilm Formation and Antibiotic Resistance

Antibiotic	Biofilm Producers Resistant (%)	Non-biofilm Producers Resistant (%)	p-value
Cefoxitin	42.5	15.7	<0.001
Ciprofloxacin	54.3	21.5	<0.001
Erythromycin	61.0	33.4	<0.001
Clindamycin	36.1	17.9	0.002

Discussion

According to this study, biofilm formation is significantly common in clinical isolates of S. aureus. The capacity of more than 60% of isolates to form biofilms highlights their possible role in persistent and chronic infections.

Biofilm production and antibiotic resistance—more especially, resistance to cefoxitin, ciprofloxacin, erythromycin, and clindamycin—were found to be significantly correlated. MRSA was more frequently found in biofilm-forming isolates, suggesting that methicillin resistance and biofilm formation may cooperate to increase bacterial survival in clinical settings [8].

The presence of latent persisting cells, altered microenvironments, and limited antibiotic penetration all contribute to the bacteria's decreased sensitivity in biofilms [9]. These results emphasize how important it is for clinical microbiology labs to regularly check for biofilm formation.

Limitations

The molecular characterisation of biofilm-associated genes (icaADBC) was not conducted. The research was performed at a single facility, perhaps constraining its generalizability.

Conclusion

Clinical isolates of Staphylococcus aureus frequently form biofilms, which are strongly associated with increased drug resistance. Early detection of S. Aureus strains that form biofilms may help guide appropriate antibiotic treatment and infection prevention strategies, improving therapeutic outcomes.

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