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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: Bacteria can establish biofilms in both natural and clinical settings on biotic and abiotic surfaces. The bacterial clumps within biofilms are constituted by the extracellular matrix they create. Staphylococcus aureus (*S. aureus*) is a prevalent bacterium associated with biofilm infections. Staphylococcal infections provide considerable therapeutic difficulties owing to antibiotic resistance facilitated by biofilms. This work defines the biofilm generation and antimicrobial resistance profiles of Staphylococcus isolates obtained from various clinical samples in a tertiary care hospital, focusing specifically on the resistance pattern discrepancies between biofilm-producing and non-producing bacteria.

Methods: We performed a laboratory-based cross-sectional study examining 100 consecutive, non-duplicate Staphylococcus isolates (44 from wound swabs, 26 from blood, 18 from urine, and 12 from respiratory samples) collected between January 2025 and December 2025. Biofilm formation was identified using the microtiter plate (MTP) method. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method in accordance with CLSI 2023 criteria.

Results: Among 100 clinical Staphylococcus infection isolates, 62% demonstrated biofilm production, with notable variation across sample types: wound swab samples showed the highest prevalence (56%, 25/44), followed by urine (50%, 9/18), respiratory samples (41.7%, 5/12), and blood samples (34.6%, 9/26). Biofilm-producing strains exhibited significantly greater antibiotic resistance compared to non-producers, particularly to erythromycin (61% vs. 33.4%, $p<0.001$).

Conclusions: Our findings indicate that biofilm-producing Staphylococcus isolates display markedly elevated resistance rates to first-line antibiotics such as ampicillin and cotrimoxazole in comparison to non-producers. The significant incidence of biofilm generation (62%) alongside the rising vancomycin resistance (0% among producers) highlights the necessity for: Regular biofilm assessment in chronic infections, Preference for linezolid/teicoplanin in instances linked with biofilms. Improved antimicrobial stewardship is essential to guide appropriate empirical therapy, optimize antibiotic selection, reduce unnecessary antibiotic use, and limit the emergence of antimicrobial resistance. These findings offer essential insights for the management of biofilm-mediated Staphylococcal infections in clinical environments.

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

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Introduction

Staphylococcus aureus is a significant human pathogen that causes a diverse array of infections, from superficial skin and soft tissue infections to severe illnesses such as bacteraemia, endocarditis, pneumonia, and osteomyelitis [1]. The therapeutic management of S. aureus infections has grown progressively difficult due to the rise of antibiotic-resistant strains, notably methicillin-resistant S. aureus (MRSA) [2], [3].

Biofilm development is a primary virulence factor that contributes to the persistence and treatment failure of *S. aureus* infections. Biofilms are organised assemblages of bacterial cells encased in a self-generated extracellular polymeric matrix that attaches to living or non-living surfaces [4]. Bacteria within biofilms display modified metabolic states and diminished vulnerability to host immunological responses and antimicrobial drugs [5].

Clinical isolates of *S. aureus* that can form biofilms are frequently linked to chronic and device-related illnesses [6]. Numerous studies indicate a significant correlation between biofilm formation capability and heightened antibiotic resistance; yet, this association differs among geographical regions and clinical contexts [7]. This study aims to assess biofilm formation in clinical isolates of *S. aureus* and to ascertain its relationship with antibiotic resistance patterns.

Methods

Study Design and Bacterial Isolates: This cross-sectional laboratory-based investigation was undertaken at Netaji Subhas Medical College and Hospital in Jamshedpur, 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	88 (88.0)firs
Cefoxitin (MRSA)	34 (34.0)
Erythromycin	49 (48.7)
Clindamycin	30 (30.0)
Ciprofloxacin	43 (42.7)
Gentamicin	23 (23.3)
Linezolid	0 (0)
Vancomycin	0 (0)

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

This study reveals a significant incidence of biofilm formation in clinical isolates of *S. aureus*. Over 60% of isolates had the ability to form biofilms, underscoring their potential involvement in chronic and persistent infections.

A notable correlation was identified between biofilm formation and antibiotic resistance, specifically to cefoxitin, ciprofloxacin, erythromycin, and clindamycin. Isolates that produce biofilm were more commonly MRSA, indicating that biofilm development and methicillin resistance may work together to improve bacterial survival in clinical settings [8].

The diminished susceptibility of bacteria within biofilms is due to restricted antibiotic penetration, modified microenvironments, and the existence of latent persister cells [9]. These findings highlight the significance of regular screening for biofilm formation in clinical microbiology laboratories.

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

Biofilm development is common in clinical isolates of Staphylococcus aureus and is closely linked to heightened antibiotic resistance. Early identification of biofilm-forming *S. Aureus* strains may assist in directing suitable antibiotic medication and infection control measures, therefore enhancing therapeutic results.

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