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Detection of Biofilm Production and Antibiotic Susceptibility Pattern of Staphylococci from Various Clinical Specimens at a Tertiary Care Hospital

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ABSTRACT

Background: Staphylococci are among the most common causes of healthcare-associated infections. Biofilm formation is an important virulence factor that contributes to antimicrobial resistance, persistence of infection, and treatment failure, particularly in methicillin-resistant strains.

Objectives: To determine the prevalence of biofilm production among clinical isolates of Staphylococcus aureus and coagulase-negative staphylococci (CoNS) and to study their antimicrobial susceptibility patterns with special reference to methicillin resistance.

Materials and Methods: A prospective study was conducted over a period of 18 months in a tertiary care hospital. A total of 150 clinically significant Staphylococcal isolates obtained from various clinical specimens were identified using standard microbiological methods. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method as per CLSI guidelines. Methicillin resistance was detected using the cefoxitin disc. Biofilm production was assessed by the tissue culture plate (TCP) method.

Results: Of the 150 isolates, 87 (58%) were Staphylococcus aureus and 63 (42%) were CoNS. Methicillin resistance was observed in 86 (57.3%) isolates, with MRSA accounting for 57.5% of S. aureus. Biofilm production was detected in 69 (46%) isolates, comprising 10 (6.7%) strong and 59 (39.3%) moderate biofilm producers. Biofilm production was significantly higher among methicillin-resistant isolates (70.9%) compared to methicillin-sensitive isolates (12.5%) ($p < 0.002$). Biofilm-producing isolates showed higher resistance to commonly used antibiotics such as penicillin, erythromycin, ciprofloxacin, and cotrimoxazole. All isolates were susceptible to vancomycin and linezolid.

Conclusion: Biofilm production is common among clinical Staphylococcal isolates and is strongly associated with methicillin resistance and multidrug resistance. Routine detection of biofilm formation along with antimicrobial susceptibility testing is essential for effective management of Staphylococcal infections and for strengthening infection control practices in hospital settings.

Keywords: *Staphylococcus aureus, Coagulase-negative staphylococci, Biofilm, Methicillin resistance, Antibiotic susceptibility.*

INTRODUCTION

Staphylococci are among the most frequently isolated Gram-positive cocci from clinical specimens and constitute a major cause of both community-acquired and healthcare-associated infections worldwide [1]. *Staphylococcus aureus* is an important human pathogen as well as a commensal organism, colonizing approximately 30% of the healthy population,

particularly the anterior nares [2]. Colonization with *S. aureus* is a well-recognized risk factor for subsequent infection and is associated with a significantly increased risk of invasive disease, especially in hospitalized and critically ill patients [3]. *S. aureus* is a leading cause of skin and soft tissue infections, surgical site infections, bloodstream infections, pneumonia, osteomyelitis, endocarditis, and device-related infections [4].

Coagulase-negative staphylococci (CoNS), previously regarded as non-pathogenic commensals of human skin and mucous membranes, have emerged as important opportunistic pathogens over the past few decades [5]. They are now recognized as major causative agents of infections associated with indwelling medical devices such as intravascular catheters, prosthetic valves, orthopedic implants, and cerebrospinal fluid shunts [6]. The increasing clinical significance of CoNS is largely attributed to their ability to adhere to biomaterial surfaces and form biofilms, which enhance their survival and persistence in the host [7].

Biofilms are structured communities of microbial cells enclosed within a self-produced extracellular polymeric matrix and adherent to biotic or abiotic surfaces [8]. Biofilm formation is a critical virulence factor in staphylococcal infections, particularly those associated with medical devices. The extracellular matrix, composed mainly of polysaccharides, proteins, and extracellular DNA, protects bacteria from host immune responses and significantly reduces susceptibility to antimicrobial agents [9]. Biofilm-associated bacteria can withstand antibiotic concentrations several times higher than those required to inhibit planktonic cells, leading to chronic, recurrent, and difficult-to-treat infections [10].

Among staphylococci, biofilm formation is mediated by several surface proteins and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), as well as the production of polysaccharide intercellular adhesin (PIA), encoded by the *ica* operon [11]. These mechanisms facilitate initial adherence, intercellular aggregation, and maturation of biofilms on medical devices and host tissues [12]. The clinical relevance of biofilm formation is evident in infections such as catheter-related bloodstream infections, prosthetic joint infections, infective endocarditis, and chronic wound infections [13].

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has further complicated the management of staphylococcal infections. Methicillin resistance is mediated by the *mecA* gene, which encodes an altered penicillin-binding protein (PBP2a) with low affinity for β-lactam antibiotics [14]. MRSA strains are often associated with multidrug resistance, limiting therapeutic options and increasing morbidity, mortality, and healthcare costs [15]. Similarly, methicillin-resistant CoNS are increasingly isolated from hospital settings and are often implicated in device-related infections [16].

Several studies have demonstrated a strong association between methicillin resistance and biofilm production in staphylococcal isolates [17]. Biofilm-producing strains are more likely to exhibit resistance to commonly used antibiotics such as erythromycin, clindamycin, fluoroquinolones, and cotrimoxazole, making treatment particularly challenging [18]. Although vancomycin and linezolid remain effective against most staphylococcal isolates, reports of reduced susceptibility and therapeutic failures highlight the need for continuous surveillance [19].

Various phenotypic methods have been described for the detection of biofilm production, including Congo red agar method, tube method, and tissue culture plate (TCP) method. Among these, the TCP method is considered the gold standard due to its quantitative nature, reproducibility, and higher sensitivity [20]. Early detection of biofilm-producing strains, along with accurate antimicrobial susceptibility testing, is essential for guiding appropriate therapy and implementing effective infection control measures [21].

In this context, the present study was undertaken to determine the prevalence of biofilm production among *Staphylococcus aureus* and coagulase-negative staphylococci isolated from various clinical specimens in a tertiary care hospital. The study also aimed to evaluate the antimicrobial susceptibility pattern of these isolates and to assess the association between biofilm production and methicillin resistance.

MATERIALS AND METHODS

Study Design and Setting

A prospective laboratory-based study was carried out in the Department of Microbiology of a tertiary care teaching hospital over a period of February 2021 to August 2022.

Sample Size and Specimens

A total of 150 clinically significant Staphylococcal isolates were obtained from specimens such as blood, pus, urine, swabs, body fluids, catheter tips, and endotracheal aspirates from critically ill patients.

Identification of Isolates

Isolates were identified based on colony morphology, Gram staining, and standard biochemical tests including catalase, coagulase, mannitol fermentation, DNase, and other relevant tests for speciation of CoNS.

Antimicrobial Susceptibility Testing
Antibiotic susceptibility testing was performed on Mueller–Hinton agar by Kirby–Bauer disk diffusion method according to CLSI guidelines. Cefoxitin disc (30 µg) was used for detection of methicillin resistance. Inducible clindamycin resistance was detected by D-test. Vancomycin minimum inhibitory concentration (MIC) was determined using E-test strips.

Detection of Biofilm Production
Biofilm formation was detected by the tissue culture plate (TCP) method using trypticase soy broth with glucose. Based on optical density readings, isolates were categorized as biofilm producers or non-producers.

Statistical Analysis
Data were compiled and analyzed using descriptive statistics. Association between biofilm production and methicillin resistance was evaluated.

RESULTS
A total of 150 clinically significant *Staphylococcal* isolates obtained from various clinical specimens during the study period were included in the analysis. The results were evaluated with respect to species distribution, sample-wise occurrence, antimicrobial susceptibility patterns, methicillin resistance, and biofilm production.

Table 1. Distribution of Staphylococcal Isolates (n = 150)

Staphylococcal group	Number of isolates	Percentage (%)
Staphylococcus aureus	87	58.0
Coagulase-negative Staphylococci (CoNS)	63	42.0
Total	150	100

Table 2. Frequency of Staphylococcal Species Isolated

Species	Number of isolates	Percentage (%)
S. aureus	87	58.0
S. haemolyticus	32	21.3
S. epidermidis	19	12.7
S. saprophyticus	7	4.7
S. capitis	2	1.3
S. lugdunensis	2	1.3
S. simmulans	1	0.7

Table 3. Sample-wise Distribution of Staphylococcal Species (n = 150)

Species	Blood	Central line	ET aspirate	Orthopedic devices	Pus	Urine	Wound	Total n (%)
S. aureus	14	6	9	2	41	8	7	87 (58.0)
S. lugdunensis	0	0	0	0	2	0	0	2 (1.3)
S. capitis	1	0	0	0	1	0	0	2 (1.3)
S. epidermidis	2	4	4	1	5	2	1	19 (12.7)
S. haemolyticus	3	7	9	3	6	3	1	32 (21.3)
S. saprophyticus	0	0	0	0	0	7	0	7 (4.7)
S. simmulans	0	0	0	0	1	0	0	1 (0.7)
Total	20	17	22	6	56	20	9	150 (100)

Table 4. Antibiotic Susceptibility Pattern of Staphylococcal Isolates (n = 150)

Antibiotic	Sensitive n (%)	Resistant n (%)
Penicillin	35 (23.3)	115 (76.7)
Amoxicillin	40 (26.7)	110 (73.3)
Cotrimoxazole	60 (40.1)	90 (59.9)
Erythromycin	69 (46.0)	81 (54.0)
Ciprofloxacin	79 (52.7)	71 (47.3)
Gentamicin	82 (54.6)	68 (45.4)
Chloramphenicol	82 (54.6)	68 (45.4)
Clindamycin	92 (61.3)	58 (38.7)
Tetracycline	103 (68.7)	47 (31.3)
Doxycycline	133 (88.7)	17 (11.3)
Linezolid	150 (100)	0 (0)
Vancomycin	150 (100)	0 (0)

Table 5. Methicillin Resistance among Staphylococcal Isolates

Isolate	Methicillin-resistant n (%)	Methicillin-sensitive n (%)
S. aureus	50 (57.5)	37 (42.5)
CoNS	36 (57.1)	27 (42.9)

Table 6. Biofilm Production by Tissue Culture Plate Method

Biofilm category	Number of isolates	Percentage (%)
Strong producers	10	6.7
Moderate producers	59	39.3
Weak / Non-producers	81	54.0

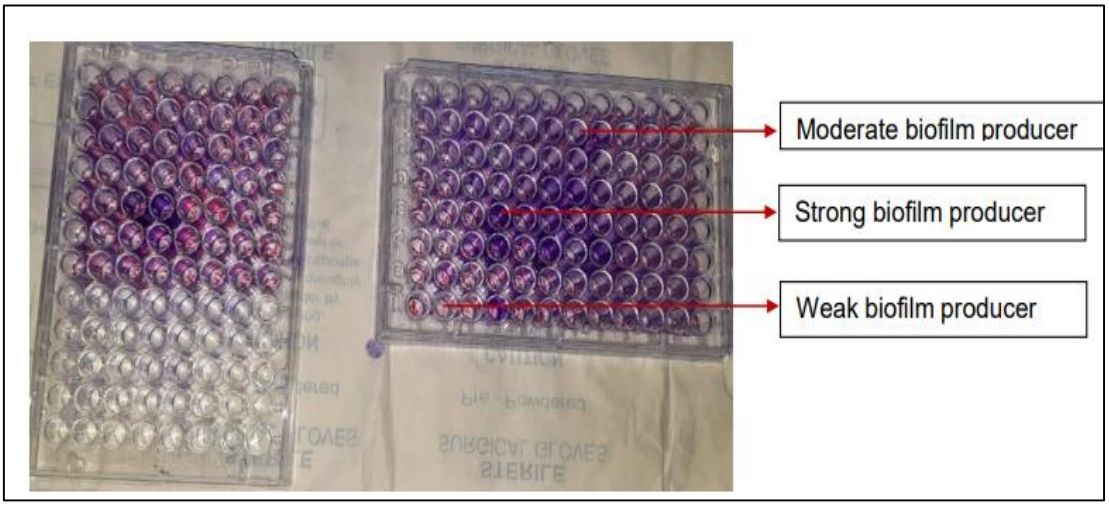

Figure 1: Tissue culture plate method showing strong ,moderate and weak biofilm production

Table 7. Biofilm Production in Relation to Methicillin Resistance

Isolate type	Methicillin-resistant (Biofilm +)	Methicillin-sensitive (Biofilm +)
S. aureus	35 / 50 (70.0%)	6 / 37 (16.2%)
CoNS	26 / 36 (72.2%)	2 / 27 (7.4%)

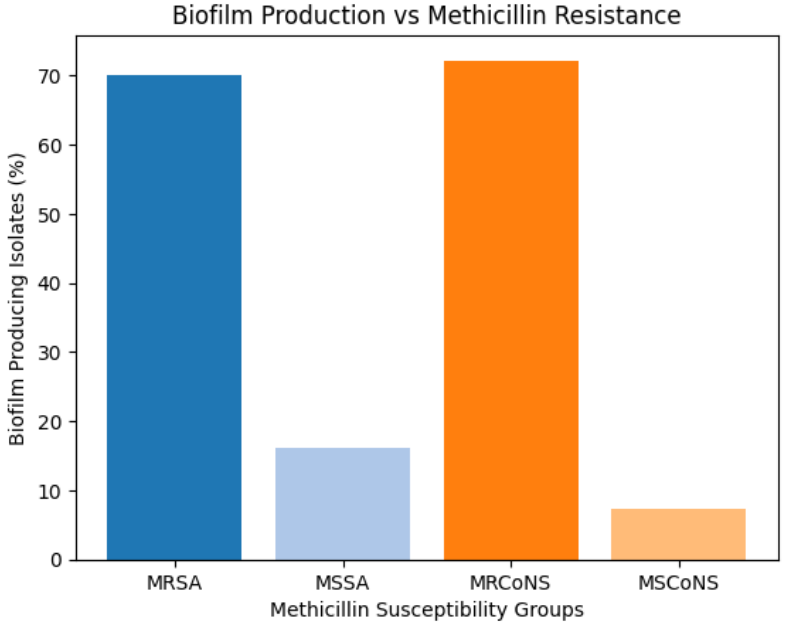

Figure 2. Biofilm production in relation to methicillin resistance among Staphylococcal isolates.

Biofilm production was significantly higher among methicillin-resistant isolates compared to methicillin-sensitive isolates. Biofilm producers constituted 70% of MRSA and 72.2% of MRCoNS isolates, whereas only 16.2% of MSSA and 7.4% of MSCoNS isolates showed biofilm production ($p < 0.002$).

DISCUSSION

Staphylococci remain one of the most important causes of healthcare-associated and community-acquired infections due to their adaptability, virulence mechanisms, and increasing antimicrobial resistance [1]. In the present study, a total of 150 clinically significant Staphylococcal isolates were analyzed to evaluate biofilm production and antimicrobial susceptibility patterns, with special emphasis on methicillin resistance.

In our study, *Staphylococcus aureus* constituted the majority of isolates (58%), followed by coagulase-negative staphylococci (42%). This finding is consistent with several Indian and international studies that have reported *S. aureus* as the predominant Staphylococcal pathogen isolated from clinical specimens [2,3]. Among CoNS, *S. haemolyticus* (50.7%) was the most frequently isolated species, followed by *S. epidermidis*. Similar observations were made by Abdel Halim et al. and other investigators, highlighting the increasing clinical relevance of *S. haemolyticus* in nosocomial infections [4,5]. The predominance of *S. haemolyticus* may be attributed to its higher antimicrobial resistance and enhanced ability to survive in hospital environments [6].

Methicillin resistance was detected in 57.3% of all Staphylococcal isolates in the present study, with MRSA accounting for 57.5% of *S. aureus* isolates. This prevalence is comparable to studies by Parasa et al. and Oberoi et al., but higher than reports by Datta et al., indicating a rising trend of MRSA in tertiary care settings in India [7–9]. The wide variation in MRSA prevalence across studies may be due to differences in patient population, infection control practices, antibiotic usage patterns, and geographic factors [10].

Biofilm production was detected in 46% of Staphylococcal isolates using the tissue culture plate (TCP) method, with 6.7% strong and 39.3% moderate biofilm producers. These findings are in concordance with studies by Mathur et al., Sharvari and Chitra, and Mohamed et al., who reported biofilm production rates ranging from 43% to 54% [11–13]. However, higher rates have been reported by Hassan et al. and Abdel Halim et al., possibly due to differences in sample selection and methodology [4,14]. The TCP method was found to be reliable and reproducible, supporting its use as a screening method for biofilm detection in routine laboratories.

A significant observation in this study was the strong association between biofilm production and methicillin resistance. Biofilm production was detected in 70.9% of methicillin-resistant Staphylococci compared to only 12.5% of methicillin-sensitive strains. Among MRSA and MRCoNS, 70% and 72.2% respectively were biofilm producers, whereas biofilm production among MSSA and MSCoNS was markedly lower. These findings are consistent with previous studies that demonstrated higher biofilm-forming capacity among methicillin-resistant strains [15–17]. The presence of biofilm confers a survival advantage by limiting antibiotic penetration and facilitating horizontal gene transfer, thereby contributing to multidrug resistance [18].

The antimicrobial susceptibility pattern observed in this study revealed high resistance rates to commonly used antibiotics such as penicillin (76.7%), amoxicillin (73.3%), cotrimoxazole (59.9%), erythromycin (54%), and ciprofloxacin (47.3%). Similar resistance trends have been reported in other studies, reflecting the widespread and often inappropriate use of these antibiotics [19,20]. Biofilm-producing isolates demonstrated higher resistance to multiple antibiotic classes, further complicating therapeutic management.

All isolates in the present study were susceptible to vancomycin and linezolid, which is in agreement with several Indian studies [21,22]. Although no vancomycin-resistant *Staphylococcus aureus* (VRSA) or vancomycin-intermediate *S. aureus* (VISA) strains were detected, the emergence of reduced susceptibility reported elsewhere underscores the importance of judicious use of glycopeptides and continuous monitoring of vancomycin MIC values [23].

Inducible clindamycin resistance was observed in 7.3% of isolates, while constitutive MLSB resistance was noted in 38.7%. These findings are comparable to reports by Upadhya et al. and Gupta et al. [24,25]. Routine performance of the D-test is essential to avoid clindamycin treatment failure, particularly in serious Staphylococcal infections.

Overall, the findings of the present study emphasize that biofilm production plays a critical role in the pathogenesis and antimicrobial resistance of Staphylococcal infections. The strong association between biofilm formation and methicillin resistance highlights the need for routine screening of biofilm production in clinical isolates, especially in hospital settings where device-related infections are common.

CONCLUSION

Biofilm production is common among clinical Staphylococcal isolates and is strongly associated with methicillin resistance and multidrug resistance. Routine screening for biofilm production along with antimicrobial susceptibility testing should be incorporated into clinical microbiology practice to guide appropriate therapy and strengthen infection control measures.

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