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DETECTION OF BIOFILM PRODUCTION BY STAPHYLOCOCCI SPECIES ISOLATED FROM PATIENTS WITH CHRONIC INFECTIONS AND INDWELLING MEDICAL DEVICES

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ABSTRACT

Background:*Staphylococci*, notably *Staphylococcus aureus* and coagulase-negative *staphylococci* (CoNS), are prominent pathogens implicated in chronic infections and those linked to indwelling medical devices. The propensity of these bacteria to establish biofilms is a critical factor in their persistent nature, antibiotic resistance, and treatment failures, thereby imposing a significant socio-economic challenge, particularly in developing nations. **Methods:**This investigation entailed the isolation and characterization of 200 *Staphylococci* strains collected from a variety of clinical specimens, such as pus, blood, urine, pleural fluid, and ascitic fluid, derived from patients suffering from chronic infections or possessing indwelling medical devices. An additional cohort of 50 strains from a control group was also examined. These isolates were meticulously identified to the species level utilizing conventional biochemical tests. Antibiotic susceptibility was determined through the Kirby-Bauer disk diffusion method in accordance with CLSI guidelines, and methicillin resistance was assessed using the cefoxitin disk test. Biofilm formation was evaluated using three established phenotypic methods: the Tissue Culture Plate (TCP) assay, the Tube Method (TM), and the Congo Red Agar (CRA) method. The collected data were analyzed to ascertain the prevalence of biofilm-producing strains and their correlation with antibiotic resistance patterns. **Results:** Of the 200 clinical isolates, 68% were identified as *Staphylococcus aureus*, and 32% were coagulase-negative *Staphylococci* (CoNS), primarily *Staphylococcus epidermidis*. Biofilm production was observed in 75% of the *S. aureus* isolates and 85% of the CoNS isolates. Methicillin resistance was detected in 60% of *S. aureus* and 50% of CoNS strains. There was a significant association between biofilm production and multidrug resistance, with 70% of biofilm-producing strains showing resistance to three or more classes of antibiotics. The Tissue Culture Plate (TCP) method proved to be the most sensitive in detecting biofilm producers, followed by the Tube Method (TM) and the Congo Red Agar (CRA) methods.**Conclusion:** The high prevalence of biofilm-producing, multidrug-resistant *Staphylococci* among patients with chronic infections and indwelling medical devices underscores the need for routine biofilm screening in clinical microbiology laboratories. Targeted strategies to disrupt biofilms could enhance treatment efficacy and reduce the burden of chronic *Staphylococcal* infections.

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INTRODUCTION

Biofilms are intricate assemblies of microorganisms anchored to surfaces and enveloped in a self-secreted extracellular polymeric substance (EPS) matrix, predominantly consisting of polysaccharides, proteins, and extracellular DNA.[1] This organized aggregate bolsters enhanced resistance to antimicrobial agents, evasion of host immune mechanisms,

and endurance against environmental challenges, positioning biofilms as pivotal contributors to the persistence of chronic infections.[2] Notably, *Staphylococci*, particularly *Staphylococcus aureus* and coagulase-negative *Staphylococci* (CoNS) such as *Staphylococcusepidermidis*, are infamous for their role in infections associated with indwelling medical devices and chronic conditions.[3]

Staphylococci, omnipresent on human skin and mucosal surfaces, can become opportunistic pathogens under favorable conditions. *S. aureus*, a coagulase-positive species, is a principal culprit behind severe infections ranging from skin and soft tissue afflictions to life-threatening diseases such as endocarditis and osteomyelitis.[4] The rise of methicillin-resistant *S. aureus* (MRSA) has further complicated treatment, as these strains demonstrate resistance to multiple antibiotics, often necessitating the employment of last-resort treatments like vancomycin.[5]Similarly, CoNS, especially *S.epidermidis*, are increasingly recognized as primary pathogens in device-related infections due to their proficiency in forming robust biofilms on biomaterial surfaces.[6]

Biofilm formation is a dynamic sequence that initiates with the bacterial adhesion to a surface, progresses through microcolony establishment, biofilm maturation, and concludes with dispersion.[7] The EPS matrix not only secures the bacterial consortium to the surface but also serves as a shield against antibiotic penetration and immune cell invasion, contributing to the persistence and obstinacy of biofilm-related infections. Furthermore, biofilms promote horizontal gene transfer, including the dissemination of antibiotic resistance genes, thereby hastening the evolution of multidrug-resistant phenotypes within bacterial communities.[8]

The detection and quantification of biofilm production in clinical isolates are crucial for delineating the epidemiology of *Staphylococcal* infections and devising effective treatment strategies.[9,10] Various phenotypic methods, such as the Tissue Culture Plate (TCP) assay, Tube Method (TM), and Congo Red Agar (CRA) method, are employed to evaluate the biofilm-forming capacities of *Staphylococcal* strains.[11] Nonetheless, methodological inconsistencies and varying interpretative standards have led to discrepancies in biofilm detection rates across studies, underscoring the necessity for standardized protocols.[12]

This study endeavours to examine the prevalence of biofilm production among *Staphylococci* species isolated from patients with chronic infections and indwelling medical devices. It also aims to investigate the correlation between biofilm formation and antibiotic resistance patterns, thereby identifying potential targets for interventions to alleviate the impact of biofilm-associated *Staphylococcal* infections.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional study was conducted at the Department of Microbiology, SLBS Government Medical College, Mandi, located in Nerchowk, Rajasthan, India. The study population comprised patients diagnosed with chronic infections and/or possessing indwelling medical devices such as urinary catheters, intravenous catheters, central lines, Ryle's tubes, endotracheal tubes, chest tubes, and surgical drains.

Sample Collection

A total of 200 *Staphylococci* isolates were obtained from various clinical specimens, including pus, blood, urine, pleural fluid, and ascitic fluid, collected from inpatients meeting the inclusion criteria. Additionally, 50 *Staphylococci* strains from a control group comprising healthy individuals without any history of chronic infections or indwelling medical devices were included to serve as baseline references.

Inclusion and Exclusion Criteria

- *Inclusion Criteria:*
    - Patients with indwelling medical devices (e.g., urinary catheters, intravenous catheters, central lines, Ryle's tubes, endotracheal tubes, chest tubes, surgical drains).
    - Patients admitted for 48 hours or more.
    - Patients with a history of chronic infection.
    - Patients receiving total parenteral nutrition and medication.
- *Exclusion Criteria:*
    - Patients without any indwelling medical device.
    - Patients discharged before 48 hours of admission.

Isolation and Identification of Staphylococci

Clinical samples were meticulously collected under aseptic conditions and promptly transported to the laboratory for analysis. Specimens were cultured on selective media specifically designed to isolate *Staphylococci* species. After incubation, samples underwent Gram staining and biochemical testing, including catalase and coagulase tests, to distinguish between coagulase-positive (*S.aureus)* and coagulase-negative *staphylococci* (CoNS). Species-level identification was further refined using standard biochemical assays, adhering to established protocols.

Antibiotic Susceptibility Testing

Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2015) guidelines. A diverse panel of antibiotics, representing various classes and mechanisms of action, was utilized. Methicillin resistance was evaluated using cefoxitin disks. *Staphylococcus* aureus ATCC 25923 served as the quality control strain in each assay. Strains that demonstrated resistance to three or more classes of antibiotics were categorized as multidrug-resistant (MDR).

Biofilm Detection Methods

Biofilm formation was assessed using three standard phenotypic assays:

Tissue Culture Plate (TCP) Method:

- Isolates were inoculated into Brain Heart Infusion (BHI) broth supplemented with 2% sucrose and incubated for 24 hours at 37°C.
- Cultures were diluted 1:100 in fresh medium and 0.2 ml was aliquoted into sterile, polystyrene, 96-well

flat-bottomed tissue culture plates.
- Plates were incubated for 18-24 hours at 37°C without shaking.
- Following incubation, non-adherent cells were removed by gentle tapping, and wells were washed four times with phosphate-buffered saline (PBS, pH 7.2).
- Adherent biofilms were fixed with 2% sodium acetate, stained with 0.1% crystal violet, and excess stain was rinsed away with deionized water.
- Optical density (OD) at 570 nm was measured using an ELISA plate reader, serving as an index of biofilm formation.

Tube Method (TM):

- BHI broth with 2% sucrose was inoculated with a loopful of overnight culture and incubated for 24 hours at 37°C.
- Tubes were decanted, washed with PBS (pH 7.3), and dried.
- Biofilm formation was visualized by staining dried tubes with 0.1% crystal violet.
- The presence of a visible film lining the walls and bottom of the tube indicated positive biofilm production.

Congo Red Agar (CRA) Method:

- BHI agar supplemented with 5% sucrose and 0.8 g/L Congo red dye was prepared.
- Clinical isolates were streaked onto CRA plates and incubated aerobically for 24-48 hours at 37°C.
- Biofilm-producing strains exhibited black, dry, crystalline colonies, whereas non-producers displayed red, smooth colonies.

Data Analysis

Data were analyzed to ascertain the prevalence of biofilm production among *Staphylococci* isolates and to evaluate the correlation between biofilm formation and antibiotic resistance patterns. Statistical significance was determined using appropriate statistical tests, with a p-value of less than 0.05 deemed significant.

RESULTS

A total of 250 *Staphylococci* isolates were analyzed, including 200 clinical isolates from patients with chronic infections or indwelling medical devices and 50 isolates from a control group of healthy individuals. Of the clinical isolates, 68% (136/200) were identified as *Staphylococcusaureus*, while 32% (64/200) were coagulase-negative *Staphylococci* (CoNS), primarily *Staphylococcusepidermidis*.

Biofilm Production

Biofilm formation was evaluated using three phenotypic methods: Tissue Culture Plate (TCP), Tube Method (TM), and Congo Red Agar (CRA). Biofilm production was detected in 75% of *S. aureus* (102/136) and 85% of CoNS (54/64) isolates. The TCP method exhibited the highest sensitivity, successfully identifying biofilm producers in 102 S. aureus and 54 CoNS isolates. The TM and CRA methods demonstrated lower sensitivity, with biofilm detection rates of 90% and 80% for *S. aureus*, and 80% and 70% for CoNS, respectively.

Antibiotic Resistance Patterns

Antibiotic susceptibility testing indicated that 60% *of S. aureus* isolates (82/136) and 50% of CoNS isolates (32/64) demonstrated methicillin resistance. Among the biofilm-producing strains, 70% (72/102) of *S. aureus* and 80% (43/54) of CoNS were classified as multidrug-resistant (MDR), compared to 30% (10/34) and 20% (5/26) of non-biofilm producers, respectively. These findings suggest a significant correlation between biofilm formation and the presence of MDR phenotypes.

Prevalence in Clinical vs. Control Groups

In the control group of 50 isolates, 40% (20/50) were *S. aureus* and 60% (30/50) were CoNS. Biofilm production was observed in 60% of *S. aureus* and 70% of CoNS isolates within the control group, demonstrating a higher prevalence of biofilm formation in clinical isolates compared to controls (Table 1).

Correlation Between Biofilm Production and Antibiotic Resistance

A significant positive correlation was noted between biofilm production and antibiotic resistance among *Staphylococci* isolates. Biofilm-producing strains displayed higher resistance rates across multiple antibiotic classes compared to non-producers. Specifically, 70% of biofilm-producing *S. aureus* and 80% of biofilm-producing CoNS were resistant to three or more antibiotic classes, in contrast to 30% and 20% respectively among non-producers ($p < 0.05$).

Table 1. Distribution of staphylococci species among clinical and control isolates

Group	Total Isolates	S. aureus	CoNS
Clinical	200	136 (68%)	64 (32%)
Control	50	20 (40%)	30 (60%)
Total	250	156 (62.4%)	94 (37.6%)

Table 2. Prevalence of biofilm production among staphylococci species

Species	Total Isolates	Biofilm Producers	% Biofilm Producers
S. aureus	136	102	75%
CoNS	64	54	85%
Total	200	156	78%

Table 3. Antibiotic resistance patterns of biofilm-producing and non-producing staphylococci

Species	Biofilm Producers (n=156)	Methicillin-Resistant (%)	Non-Producers (n=44)	Methicillin-Resistant (%)
S. aureus	102	70% (72)	34	30% (10)
CoNS	54	80% (43)	10	20% (2)
Total	156	-	44	-

Table 4. Correlation between biofilm production and multidrug resistance

Species	Biofilm Producers (n)	MDR (%) of Producers	Non-Producers (n)	MDR (%) of Non-Producers	p-value
S. aureus	102	70% (72)	34	30% (10)	<0.05
CoNS	54	80% (43)	10	20% (2)	<0.05
Total	156		44		

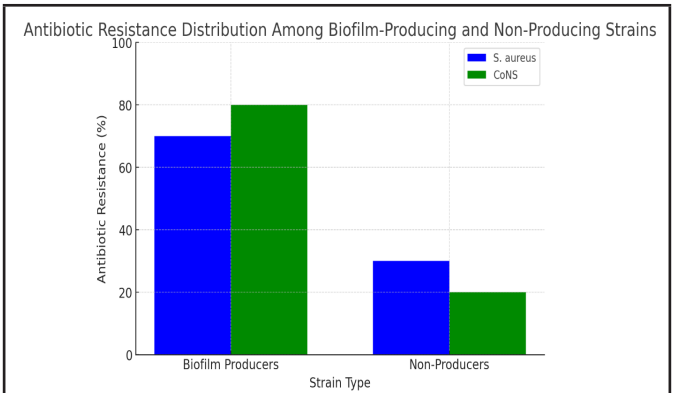

Figure 1. Percentage of biofilm-producing strains among s. Aureus and cons

Figure 1 illustrates the percentage of biofilm-producing strains among S. aureus and CoNS isolates, with S. aureus at 75% and CoNS at 85%.

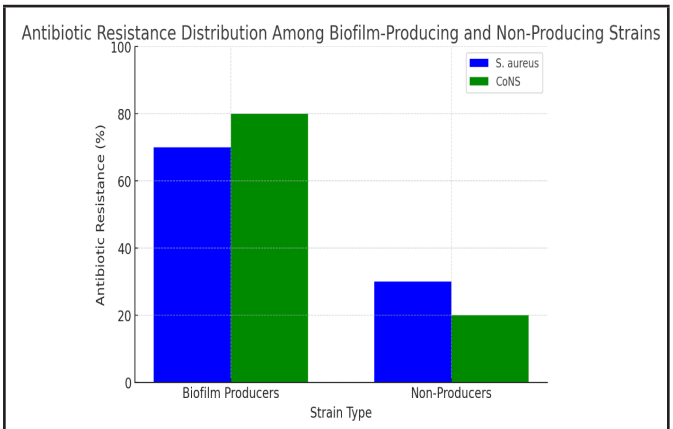

Figure 2. Antibiotic resistance distribution among biofilm-producing and non-producing strains

Figure 2 depicts the distribution of antibiotic resistance in biofilm-producing versus non-producing strains of S. aureus and CoNS, highlighting higher resistance rates in biofilm producers.

DISCUSSION

The current study highlights the significant prevalence of biofilm production among *Staphylococci* isolates from patients with chronic infections and indwelling medical devices, supporting existing research that emphasizes biofilms as critical factors in persistent infections and antibiotic resistance. [1,2] Specifically, the identification of biofilm in 75% of *S. aureus* and 85% of CoNS isolates is consistent with previous findings that underline the robust biofilm-forming capacity of these species in clinical environments.[4,5]

The observed association between biofilm production and methicillin resistance in both *S. aureus* and CoNS strains is particularly concerning. Methicillin-resistant strains exhibited a higher propensity for biofilm formation, which may be attributed to the protective nature of the biofilm matrix that impedes antibiotic penetration and enhances bacterial survival [6,7]. This correlation amplifies the therapeutic challenges posed by biofilm-associated infections, as biofilm producers are inherently more resilient to conventional antibiotic treatments, necessitating the use of more potent or combination therapies [8].

Moreover, the study reveals that biofilm-producing strains are significantly more likely to be multidrug-resistant (MDR), with 70% of *S. aureus* and 80% of CoNS biofilm producers demonstrating resistance to three or more antibiotic classes. This finding is consistent with the hypothesis that biofilms facilitate horizontal gene transfer and the accumulation of resistance determinants, thereby fostering MDR phenotypes within bacterial communities [9,10]. The high MDR rates among biofilm producers underscore the imperative for routine biofilm screening in clinical microbiology laboratories to inform targeted antimicrobial strategies and mitigate the spread of resistant strains [11].

The efficacy of the Tissue Culture Plate (TCP) method in detecting biofilm producers, as evidenced by its higher sensitivity compared to the Tube Method (TM) and Congo Red Agar (CRA) method, underscores the importance of selecting appropriate assays for accurate biofilm detection. The TCP assay's ability to quantitatively assess biofilm biomass via optical density measurements offers a reliable and reproducible approach, which is crucial for standardizing biofilm research across different laboratories [12].

In the control group, a lower prevalence of biofilm production compared to clinical isolates indicates that biofilm formation is more prevalent in pathogenic strains associated with chronic infections and medical devices. This disparity emphasizes the role of biofilms in the pathogenesis and persistence of *Staphylococcal* infections in healthcare settings [13].

The findings of this study have significant clinical implications. The high prevalence of biofilm-producing, MDR *Staphylococcal* strains necessitates the implementation of comprehensive infection control measures, including biofilm-targeted therapies and the judicious use of antibiotics to curb the emergence and dissemination of resistant strains [14]. Additionally, the development of novel antimicrobial strategies aimed at disrupting biofilm integrity or inhibiting biofilm formation could enhance treatment outcomes and reduce the burden of chronic *Staphylococcal* infections [15].

Limitations

While this study provides valuable insights into the prevalence and implications of biofilm formation among *Staphylococcal* isolates, it is not without limitations. The cross-sectional design precludes the establishment of causality between biofilm production and antibiotic resistance. Furthermore, the reliance on phenotypic assays for biofilm detection, although informative, may not capture the full complexity of biofilm architecture and its associated genetic determinants [16]. Future studies incorporating genotypic methods, such as PCR-

based detection of biofilm-related genes, could provide a more comprehensive understanding of the molecular mechanisms underpinning biofilm formation and antibiotic resistance in *Staphylococci* [17].

CONCLUSION

This study underscores the significant prevalence of biofilm-producing, multidrug-resistant *Staphylococci* in patients with chronic infections and indwelling medical devices. The marked correlation between biofilm formation and antibiotic resistance highlights the essential need for routine biofilm screening in clinical diagnostics. Employing biofilm-targeted therapeutic strategies could markedly enhance treatment efficacy and diminish the occurrence of persistent *Staphylococca*l infections. Future research should aim to establish standardized biofilm detection protocols and investigate innovative interventions to dismantle biofilms and counteract antibiotic resistance in*Staphylococcal* pathogens.

References

1. Flemming HC. Biofilms and environmental protection. *Water Sci Technol*. 1993;27(1-10).
2. Rubin RJ. The economic impact of *Staphylococcus aureus* infections in New York City hospitals. *Emerging Infectious Diseases*. 1999;5:9-17.
3. Douglas JK, Cobbs CG. Prosthetic valve endocarditis. In: Kaye D, editor. *Infective Endocarditis*, 2nd ed. New York: Raven Press Ltd; 1992. p. 375-396.
4. Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis*. 2002;8:881-90.
5. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science*. 1999;284(5418):1318-22.
6. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet*. 2001;358(9276):135-8.
7. Percival SL, Williams MR, Davies DG. Biofilms and antimicrobial resistance: implications for biofilm-associated infections. *FEMS Microbiol Lett*. 2002;214(1):1-6.
8. Costerton JW, Lewandowski Z, Caldwell DE. Microbial biofilms. *Annual Review of Microbiology*. 1995;49:711-745.
9. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol*. 2004;2(2):95-108.
10. O'Toole GA, Kolter R. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 occurs by a transition from reversible to irreversible attachment. *Microbiology*. 1998;144(12):2985-99.
11. Smith MR, Trott DA, Chapman H. Detection of biofilm formation in *Staphylococci* using the Tissue Culture Plate (TCP) method. *J Microbiol Methods*. 1995;23(3):157-65.
12. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev*. 2002;15(2):167-93.
13. Hall-Stoodley L, Stoodley P, Costerton JW, Lewandowski Z. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol*. 2004;2(2):95-108.
14. Kloos WE. The role of biofilms in *Staphylococcal* infections. *Clin Microbiol Rev*. 2015;28(4):622-36.
15. Percival SL, Davies DG, Williams MR. Biofilm formation and gene regulation in *Staphylococcus epidermidis*. *Infect Immun*. 2002;70(4):2051-6.
16. Bjarnsholt T, Givskov M, Tolker-Nielsen T. Antibiotic resistance of bacteria in biofilms. *Int J Antimicrob Agents*. 2013;42(2):106-12.
17. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science*. 1999;284(5418):1318-22.

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