RESEARCH PAPER

Phenotypic Assessment of biofilm production in Staphylococcal isolates from clinical specimens and their antibiotic susceptibility pattern at a tertiary care hospital in Central India

Mohammad Asif Nagori[1], Dr Munesh Kumar Sharma[2], Pragati Patel[3], Dr Nidhi Singh[4]

[1,3]PhD Scholar, Department of Microbiology, Index Medical College, Hospital and Research Centre, Malwanchal University, Indore, India

[2]Professor, Department of Microbiology, Index Medical College, Hospital and Research Centre, Malwanchal University, Indore, India

[4]Senior Resident, Department of Microbiology, Rama Medical College, Kanpur, UP, India.

ABSTRACT

BACKGROUND

Biofilm formation is an important survival mechanism used by microorganisms to attach to living and non-living surfaces. Once formed, these biofilms protect bacteria from host immune responses and reduce the effectiveness of antimicrobial agents, often leading to persistent or recurrent infections, particularly in patients with wounds, catheters, or implanted medical devices. The present study was conducted to detect biofilm production among clinical staphylococcal isolates and to assess their antibiotic susceptibility pattern.

MATERIALS AND METHODS

This study was carried out in the Department of Microbiology, Index Medical College, Hospital and Research Centre, Indore, India. A total of 300 staphylococcal isolates obtained from different clinical specimens were included. All isolates were processed and identified using standard microbiological procedures. Biofilm production was detected phenotypically by the Congo Red Agar method, and antibiotic susceptibility testing was performed according to standard laboratory guidelines.

RESULTS

Among the 300 staphylococcal isolates studied, 141 were identified as Staphylococcus aureus and 159 as coagulase-negative staphylococci. Biofilm production was observed in 56 S. aureus isolates, giving a positivity rate of 39.72%. Among coagulase-negative staphylococci, 87 isolates were biofilm producers, accounting for 54.72%. Overall, 143 out of 300 isolates showed biofilm-forming ability, with an overall prevalence of 47.67%. Biofilm production was more frequent among coagulase-negative staphylococci than S. aureus.

Keywords: Staphylococcus aureus; coagulase-negative staphylococci; biofilm production; Congo Red Agar; antibiotic susceptibility.

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INTRODUCTION

Biofilms are structured communities of microorganisms that attach to living or non-living surfaces and surround themselves with a self-produced protective matrix. This matrix is mainly composed of extracellular polymeric substances, including polysaccharides, proteins, lipids, and extracellular DNA. By living within this protective environment, bacteria are able to survive adverse conditions and remain attached to tissues, catheters, implants, and other medical devices. Such attachment often marks the beginning of colonisation and may later result in clinically significant infections such as catheter-associated urinary tract infections, prosthetic valve endocarditis, and other device-related infections.[1]

Biofilm formation is an important virulence mechanism in many bacterial species. In healthcare settings, biofilms are commonly associated with chronic, recurrent, and difficult-to-treat infections. They may develop on damaged tissues, surgical wounds, indwelling catheters, prosthetic joints, pacemakers, and other implanted devices. Outside the clinical environment, biofilms also

create problems in industries by contributing to biofouling in water systems and contamination in food-processing units. Common biofilm-forming organisms include *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus viridans*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus mirabilis*.[2,3]

The ability to form biofilm is seen in both Gram-positive and Gram-negative bacteria. Infections associated with biofilms are often persistent because organisms inside the biofilm behave differently from free-floating, or planktonic, bacterial cells. They grow slowly, communicate through signalling mechanisms, and remain protected from environmental stress. As a result, biofilms are linked with several clinical conditions, including urinary tract infections, dental plaque, respiratory tract infections, peritonitis, urogenital infections, wound infections, and infections related to indwelling medical devices.[3]

One of the major concerns with biofilm-producing bacteria is their reduced susceptibility to antimicrobial agents. Bacteria enclosed within a biofilm may tolerate antibiotics at concentrations much higher than those required to inhibit planktonic cells. This reduced response is partly due to poor penetration of antibiotics through the biofilm matrix, slow bacterial growth, altered metabolism, and the presence of dormant persister cells.[3]

Biofilm formation also contributes to antimicrobial resistance through several mechanisms, including reduced cell permeability, enzyme-mediated drug modification, activation of efflux pumps, and exchange of resistance genes among closely packed bacterial cells. These factors make complete eradication of biofilm-associated infections difficult and increase the risk of treatment failure.[4,7]

In addition to limiting antibiotic activity, biofilms help microorganisms evade host immune responses. The biofilm matrix acts as a physical barrier, protecting bacteria from phagocytosis and other immune defence mechanisms. This allows the organisms to persist in the host for longer periods and may lead to chronic or recurrent infections. Because of these properties, biofilm formation plays an important role in the pathogenesis and severity of hospital-acquired infections.[5–7]

Objectives

Early detection of biofilm-producing staphylococci is important for the prevention and management of hospital-acquired infections, especially those associated with wounds, catheters, and other medical devices. The present study was designed to evaluate the biofilm-forming ability of staphylococcal isolates obtained from different clinical specimens. The study also aimed to assess the antibiotic susceptibility pattern of these isolates and to observe its association with biofilm production.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, Index Medical College, Hospital and Research Centre, Indore, India, from January 2025 to January 2026. Patients attending various outpatient and inpatient departments of the hospital were included. A total of 300 staphylococcal isolates obtained from clinical specimens were studied. Isolates were included irrespective of the patient's age, sex, occupation, religion, or ethnicity.

Sample Collection

Clinical specimens such as urine, blood, pus, wound swabs, vaginal swabs, and central line tips were collected from patients attending different OPD and IPD departments of Index Medical College, Hospital and Research Centre, Indore. All samples were collected using aseptic precautions to avoid contamination. After collection, the specimens were transported promptly to the microbiology laboratory under appropriate conditions for further processing.

Phenotypic Assay for Biofilm Detection

Congo Red Agar Method

Biofilm production was detected phenotypically using the Congo Red Agar method. The medium was prepared using Brain Heart Infusion agar supplemented with 5% sucrose and Congo red dye. The Congo red solution was prepared separately as a concentrated solution and sterilized by autoclaving at 121°C for 15 minutes. After the agar medium cooled to approximately 55°C, the Congo red solution was added slowly and mixed properly before pouring the plates. The prepared Congo Red Agar plates were inoculated with the test isolates and incubated aerobically at 37°C for 24 hours. To ensure reliability of the results, the procedure was repeated. Colony colour was observed carefully after incubation. Isolates producing black colonies, including bright black or opaque black colonies, were interpreted as biofilm producers. Colonies showing pink, red, or Bordeaux colour were considered non-biofilm producers.[2,8]

Modified Congo Red Agar Method

A modified Congo Red Agar method has also been described in previous studies to improve the detection of biofilm production, particularly in isolates where black pigment formation is inconsistent. In this modified method, the concentration of Congo red dye is reduced to 0.4 g/L, sucrose is replaced with glucose at a concentration of 10 g/L, and Blood Agar Base-2 is used instead of Brain Heart Infusion agar. These changes help provide a more stable medium and improve the visual interpretation of biofilm-producing colonies.[2,9,10]

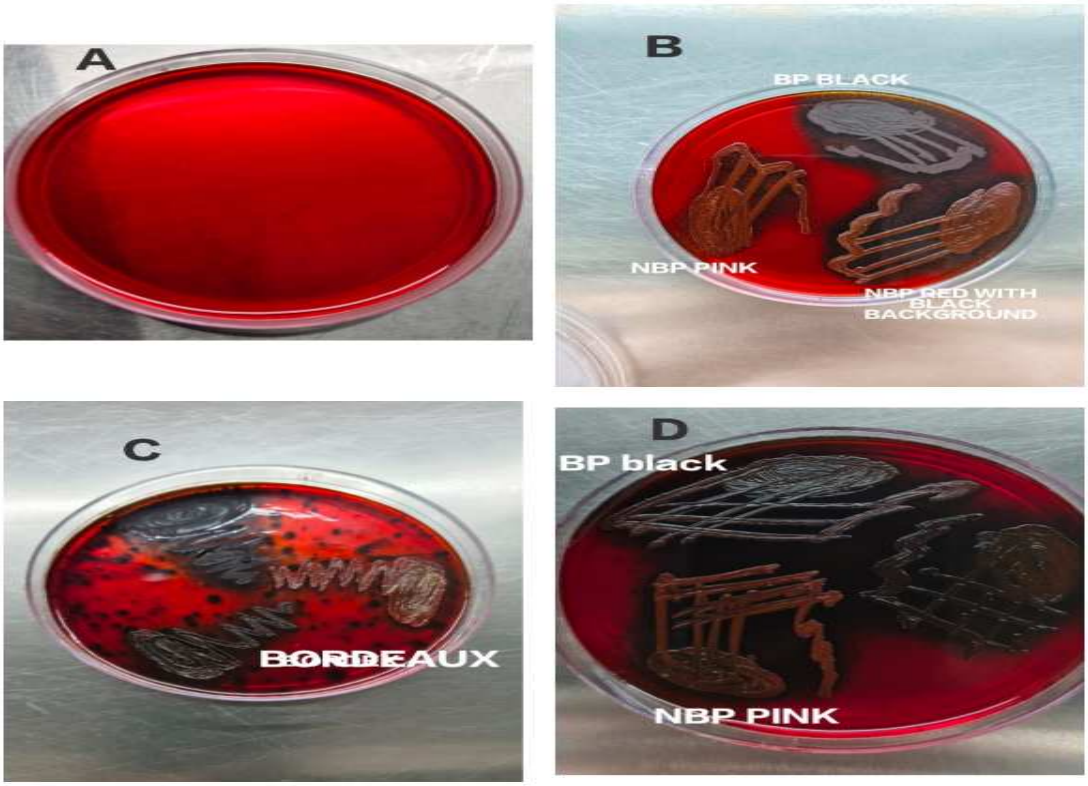


Figure 1:Variability in Biofilm-Producing Isolates Evidenced by the Congo Red Agar

Method A: uninoculated Congo red agar, B: non-biofilm producer, non-biofilm producer with black background, C: Bordeaux, D:black biofilm producer with black background

RESULT

A total sample size of 300 staphylococcal isolates, 141 were identified as *Staphylococcus aureus* (SA) and 159 as Coagulase-Negative Staphylococci (CONS). Evaluation for biofilm formation revealed that 56 of the 141 SA isolates (39.72%) were biofilm producers. In comparison, CONS demonstrated a higher rate of biofilm production, with 87 out of 159 isolates (54.72%) testing positive. In total, 143 of the 300 isolates (47.67%) were identified as biofilm producers.

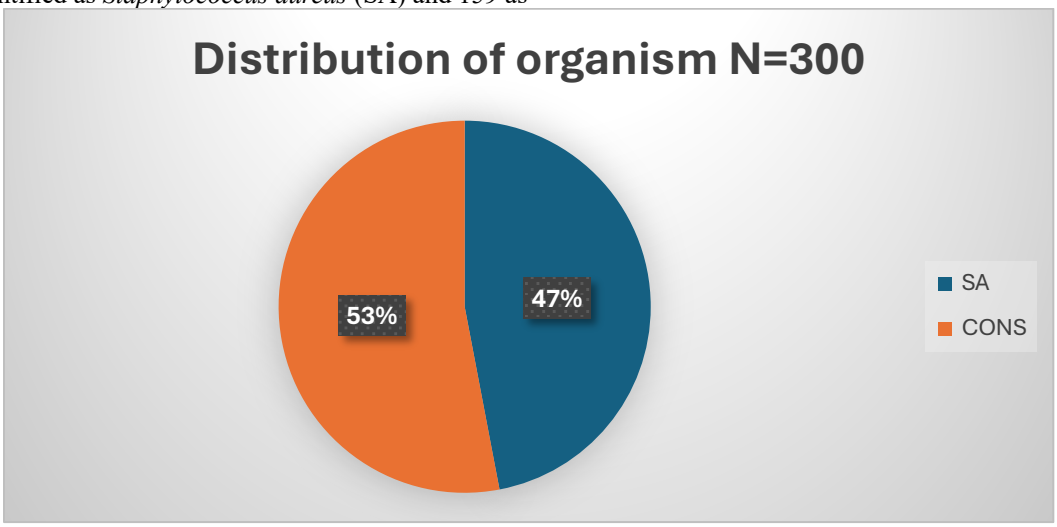


FIGURE 2 DISTRIBUTION OF ORGANISM

The distribution of biofilm producers categorised by patient gender across a total sample size of 300. Male patients accounted for the majority of the cases with 182 samples, of which 70 were identified as biofilm producers (38.46%). In contrast, female patients represented 118 samples, yielding a higher positivity rate with 73 confirmed biofilm producers (61.86%). Across both groups, a total of 143 out of 300 samples (47.67%) demonstrated biofilm production.

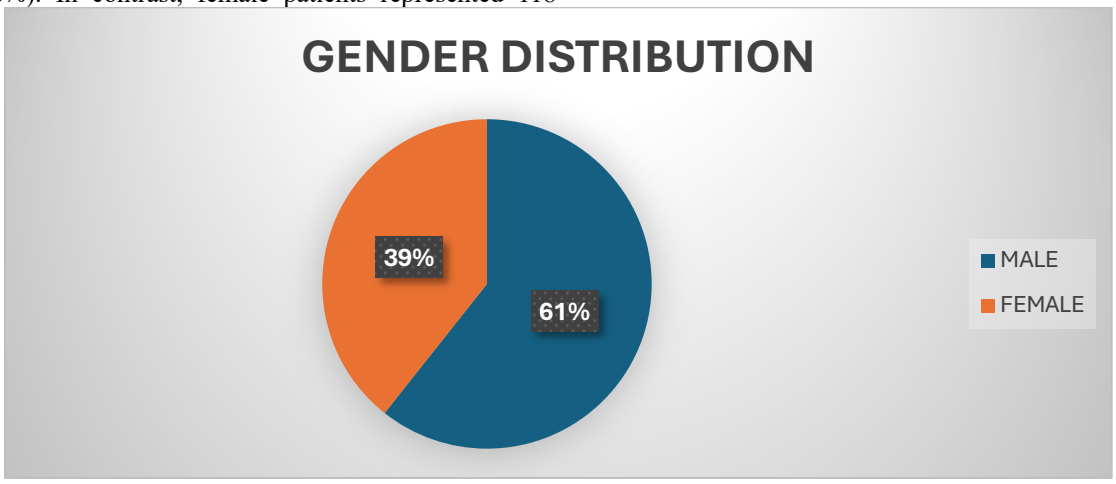


FIGURE 3 DISTRIBUTION OF GENDER

A total of 300 samples were evaluated across different specimen types to determine the presence of film producers. Pus samples constituted the largest category with 196 total specimens, of which 81 were identified as film producers. Urine followed with 32 samples (19 film producers), while blood accounted for 25 samples (9 film producers). Both vaginal swabs and wound swabs had 14 samples each, with all specimens (100%) testing positive as film producers. Additionally, 6 out of 13 central line tip samples were film producers. In contrast, none of the 6 other swab samples produced film. Overall, 143 out of the 300 samples (47.67%) were identified as film producers across the evaluated groups.

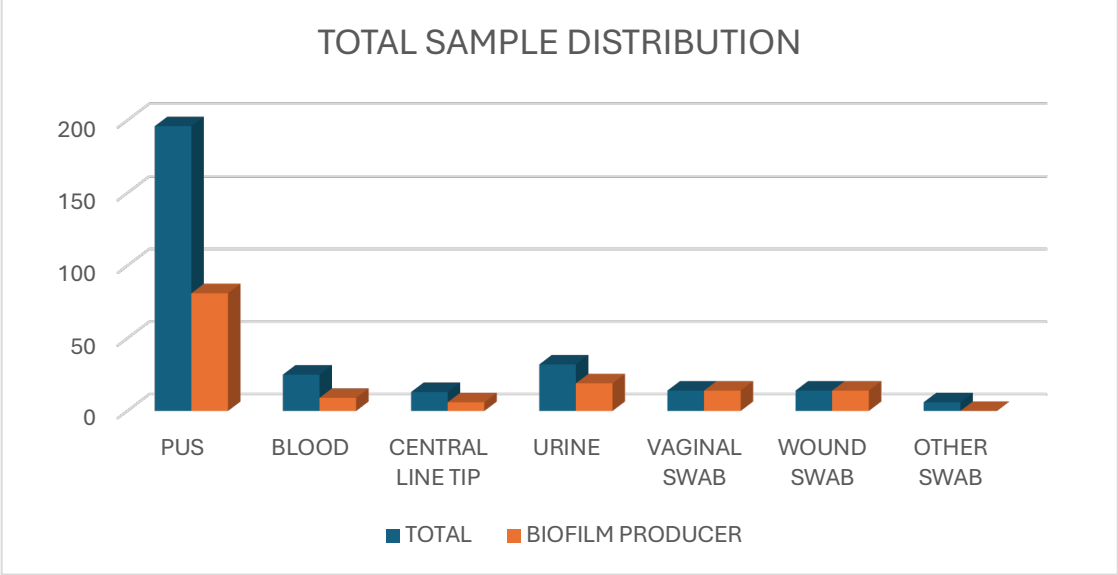


FIGURE 4 DISTRIBUTION OF SAMPLE

The distribution of total samples and biofilm producers across 11 different hospital departments, encompassing 300 total specimens. General Surgery accounted for the highest volume of samples at 96, yielding 43 biofilm producers (44.79%). Obstetrics and Gynaecology (OBS/GYNAE) contributed 52 samples, with a high yield of 40 biofilm producers (76.92%), while Dermatology (DERMA) recorded 20 producers out of 51 specimens (39.22%). Both ENT and NICU demonstrated a 100% positivity rate, with all 6 collected samples in each department identified as biofilm producers. Orthopaedics (ORTHO) showed an exact 50% positivity rate with 12

producers out of 24 samples, followed by Paediatrics (PEDIA) with 9 out of 19 (47.37%), and OPD with 7 out of 21 (33.33%). In contrast, no biofilm producers were detected in General Medicine (0 out of 6), PICU (0 out of 13), or Psychiatry (0 out of 6). Across all departments, a total of 143 out of 300 samples (47.67%) were identified as biofilm producers.

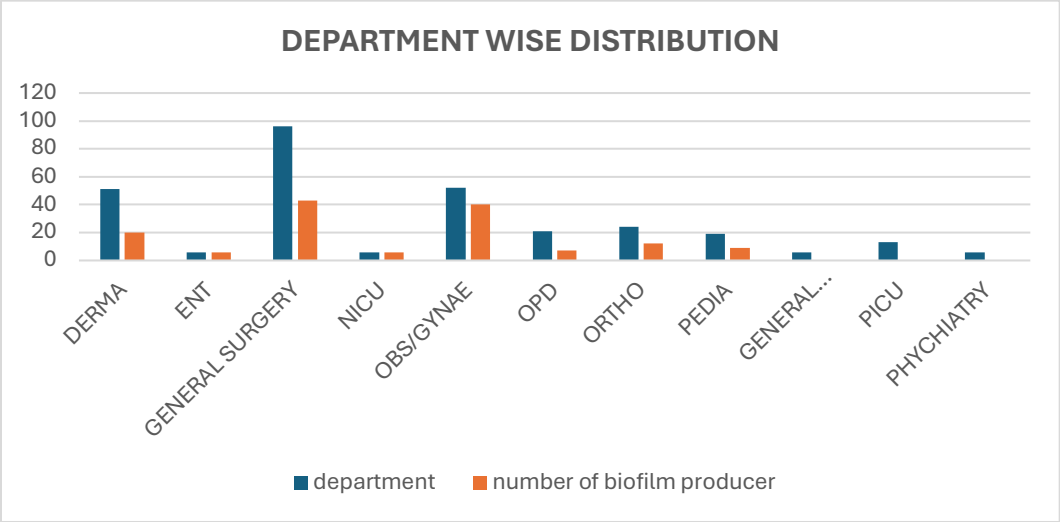


FIGURE 5 DEPARTMENT-WISE DISTRIBUTION

TABLE 1: DISTRIBUTION OF STAPHYLOCOCCUS AUREUS Regarding Gender, Department and Samples.

STAPHYLOCOCCUS AUREUS{141}	BIOFILM PRODUCER {56}	
MALE	50	
FEMALE	6	
MRSA	26	
POSITIVE SA SAMPLE DEPARTMENT-WISE N=56		
DERMA	14	PUS 14
GENERAL SURGERY	30	PUS 24, URINE 6
ENT	6	PUS 6
ORTHO	6	PUS 6

Among the 141 *Staphylococcus aureus* isolates evaluated, 56 (39.72%) were confirmed to be biofilm producers. Regarding the gender distribution among these 56 biofilm-positive isolates, male patients predominated with 50 cases (89.29%), while female patients accounted for 6 cases (10.71%). Furthermore, 26 of the 56 biofilm-producing isolates (46.43%) were identified as Methicillin-Resistant *Staphylococcus aureus* (MRSA). Departmental distribution demonstrated that General Surgery yielded the highest number of biofilm-positive *S. aureus* isolates at 30 (53.57%), comprising 24 Pus and 6 urine samples. This was followed by Dermatology with 14 isolates (25.00%), all recovered from pus specimens, while both ENT and Orthopaedics contributed 6 isolates each (10.71%), with ENT and Orthopaedics isolates originating entirely from pus samples.

TABLE 2: DISTRIBUTION OF COAGULASE NEGATIVE STAPHLOCOCCUS SPECIES REGARDING GENDER, DEPARTMENT AND SAMPLES.

CONS {159}	BIOFILM PRODUCER{87}
MALE	20
FEMALE	67
MRS	47
POSITIVE CONS SAMPLE DEPARTMENT-WISE N=87	

NICU	6	CENTRAL LINE TIP 6
GENERAL SURGERY	7	PUS 7
OBS/GYNAE	20	PUS 6, WOUND SWAB 14
OPD	7	URINE 7
PEDIATRICS	7	BLOOD 7

Among the 159 Coagulase-Negative Staphylococci (CoNS) isolates evaluated, 87 (54.72%) were identified as biofilm producers. A distinct demographic pattern was observed among these biofilm-positive isolates, with female patients accounting for the majority at 67 cases (77.01%), compared to 20 cases (22.99%) in males. In addition, 47 of the 87 biofilm-producing CoNS isolates (54.02%) demonstrated methicillin resistance (MRS). Regarding the departmental and specimen distribution of the positive isolates, Obstetrics and Gynaecology (OBS/GYNAE) recorded the highest frequency with 20 isolates, consisting of 14 wound swabs and 6 pus samples. General Surgery, OPD, and Paediatrics contributed 7 isolates each, derived respectively from pus, urine, and blood specimens, while the NICU accounted for 6 isolates, all recovered from central line tips.

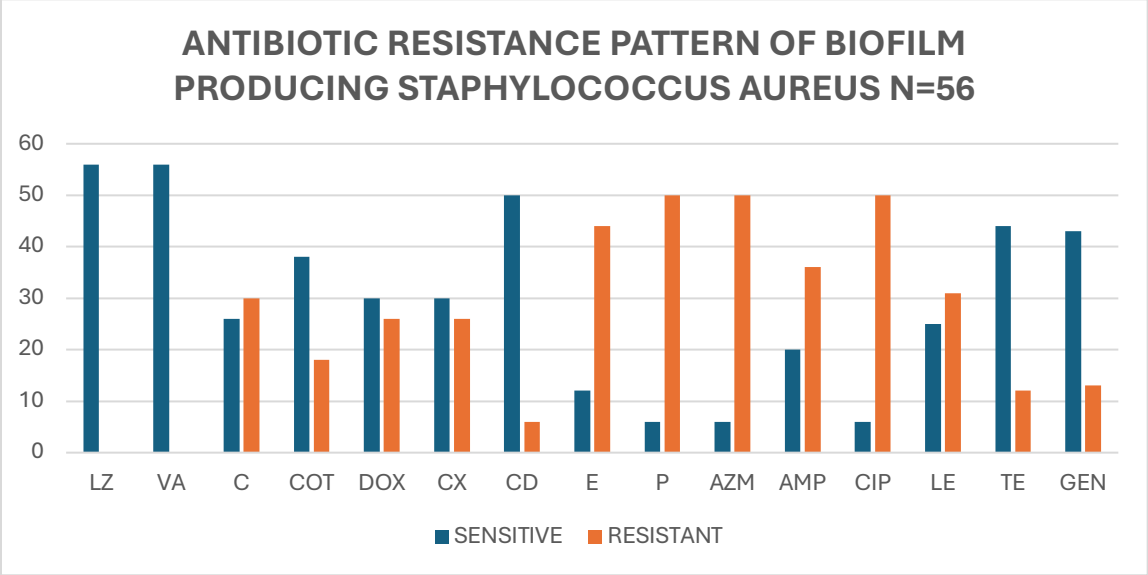


FIGURE 6 ANTIBIOTIC RESISTANCE PATTERN OF BIOFILM-PRODUCING STAPHYLOCOCCUS AUREUS

The antibiotic Resistance pattern of the 56 biofilm-producing *Staphylococcus aureus* isolates demonstrated complete sensitivity (100%, 56/56) to linezolid (LZ) and vancomycin (VA), with no resistance observed. High levels of sensitivity were also exhibited towards clindamycin (CD) at 89.29% (50 sensitive, 6 resistant), tetracycline (TE) at 78.57% (44 sensitive, 12 resistant), gentamicin (GEN) at 76.79% (43 sensitive, 13 resistant), and co-trimoxazole (COT) at 67.86% (38 sensitive, 18 resistant). Both doxycycline (DOX) and cefoxitin (CX) showed moderate susceptibility, each recording 30 sensitive (53.57%) and 26 resistant (46.43%) isolates, while chloramphenicol (C) and levofloxacin (LE) exhibited sensitivity rates of 46.43% (26/56) and 44.64% (25/56), respectively. Conversely, substantial resistance was evident against penicillin (P), azithromycin (AZM), and ciprofloxacin (CIP), each displaying 89.29% resistance (50 resistant, 6 sensitive), followed by erythromycin (E) at 78.57% resistance (44 resistant, 12 sensitive) and ampicillin (AMP) at 64.29% resistance (36 resistant, 20 sensitive).

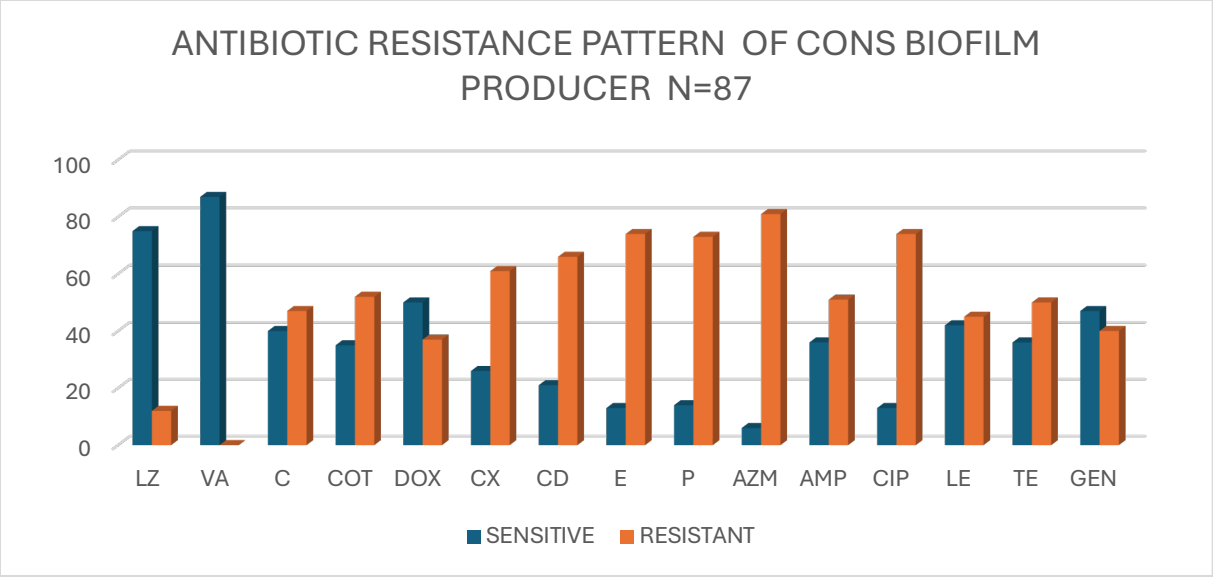


FIGURE 7 ANTIBIOTIC RESISTANCE PATTERN OF BIOFILM-PRODUCING CONS

The antibiotic Resistance pattern of the 87 biofilm-producing Coagulase-Negative Staphylococci (CoNS) isolates demonstrated complete (100%) susceptibility to vancomycin (VA: 87 sensitive, 0 resistant), followed by a high rate of sensitivity to linezolid (LZ: 75 sensitive, 12 resistant; 86.21%). Moderate susceptibility was retained against doxycycline (DOX: 50 sensitive, 37 resistant; 57.47%), gentamicin (GEN: 47 sensitive, 40 resistant; 54.02%), levofloxacin (LE: 42 sensitive, 45 resistant; 48.28%), and chloramphenicol (C: 40 sensitive, 47 resistant; 45.98%). Comparable intermediate profiles were noted for ampicillin (AMP) and tetracycline (TE), each recording 36 sensitive isolates against 51 and 50 resistant isolates, respectively, as well as co-trimoxazole (COT: 35 sensitive, 52 resistant). In contrast, pronounced resistance was observed against cefoxitin (CX: 26 sensitive, 61 resistant; 70.11%) and clindamycin (CD: 21 sensitive, 66 resistant; 75.86%), with the highest degrees of resistance recorded against penicillin (P: 14 sensitive, 73 resistant; 83.91%), erythromycin (E) and ciprofloxacin (CIP) (each with 13 sensitive, 74 resistant; 85.06%), and azithromycin (AZM: 6 sensitive, 81 resistant; 93.10%).

DISCUSSION

Staphylococci are important opportunistic pathogens and are frequently involved in both hospital-acquired and community-acquired infections. Their ability to form biofilms makes these infections more difficult to treat, especially when medical devices or damaged tissues are involved. In the present study, biofilm production was observed in 143 out of 300 staphylococcal isolates, giving an overall positivity rate of 47.67%. This finding suggests that biofilm formation is common among clinical staphylococcal isolates and may contribute to persistence of infection in a considerable number of cases.

In this study, coagulase-negative staphylococci showed a higher rate of biofilm production than *Staphylococcus aureus*. Among 159 CoNS isolates, 87 were biofilm producers, accounting for 54.72%. In comparison, 56 out of 141 *S. aureus* isolates, or 39.72%, showed biofilm-forming ability. CoNS were once considered mainly as skin commensals or contaminants, but they are now increasingly recognised as significant opportunistic pathogens, particularly in patients with indwelling medical devices. Their pathogenic role is closely related to their ability to adhere to artificial surfaces and produce an extracellular slime layer, which helps them persist on catheters, prosthetic valves, orthopaedic implants, and other biomaterials.[8,11]

The lower biofilm positivity observed among *S. aureus* isolates in this study may be related to the diverse pathogenic behaviour of this organism. Some *S. aureus* strains, especially those involved in chronic wounds, osteoarticular infections, or device-associated infections, are strong biofilm producers. However, strains associated with acute invasive infections may rely more on toxin production, tissue invasion, and dissemination rather than stable biofilm formation. This may explain why biofilm production was detected in 39.72% of *S. aureus* isolates in the present study.[12,13]

The higher biofilm-forming ability of CoNS observed in this study is consistent with their known association with device-related infections. Biofilm production allows these organisms to remain attached to surfaces and resist

removal by host defence mechanisms. Once a biofilm is established, bacteria become less accessible to antimicrobial agents, making infections more persistent and difficult to eradicate.[8,11]

Biofilm-associated staphylococcal infections are clinically important because bacteria enclosed within the biofilm matrix show reduced susceptibility to antibiotics compared with free-living planktonic cells. This reduced response may occur due to poor antibiotic penetration through the matrix, slow bacterial growth, altered metabolic activity, and the presence of dormant persister cells. As a result, infections caused by biofilm-producing staphylococci may not respond adequately to routine antibiotic therapy alone. In such cases, appropriate antimicrobial therapy, removal of infected devices when required, and proper surgical debridement are important for successful management.[8,12]

In the present study, male patients contributed a larger proportion of the total isolates, with 182 out of 300 samples obtained from males. However, biofilm positivity was proportionally higher among females. Biofilm production was detected in 73 of 118 female isolates, giving a positivity rate of 61.86%, whereas 70 of 182 male isolates were biofilm positive, with a rate of 38.46%. This difference may be related to the type of clinical specimens and infection sites rather than any direct effect of sex on biofilm formation.

Biofilm production is mainly influenced by bacterial factors such as adhesins, extracellular matrix production, and regulatory genes involved in attachment and persistence. Therefore, the higher positivity among female patients in this study may reflect greater representation of urogenital, obstetric, gynaecological, or catheter-associated specimens, where organisms such as *S. saprophyticus* and *S. epidermidis* commonly form biofilms. In contrast, many isolates from male patients were obtained from acute wound or soft-tissue infections, where bacteria may behave more as invasive planktonic cells rather than established biofilm communities.[12,15–18]

Thus, the observed gender-wise difference in biofilm production appears to be influenced mainly by sample source, anatomical site, and device exposure. These findings highlight the need for careful interpretation of biofilm data along with clinical background, specimen type, and hospital setting. Routine detection of biofilm production in clinically significant staphylococcal isolates may help guide treatment decisions and improve infection control practices.

In the present study, biofilm production was detected in 143 out of 300 staphylococcal isolates, giving an overall positivity rate of 47.67%. The rate of biofilm formation varied according to the type of clinical specimen. Vaginal swabs and wound swabs showed the highest positivity, with all 14 isolates from each group producing biofilm. This may be because these sites provide favourable conditions for bacterial attachment and persistence, especially in the presence of mucosal surfaces, tissue injury, secretions, or foreign material such as sutures and catheters.

Pus was the most common specimen in this study, accounting for 196 out of 300 isolates. Among these, 81 isolates were biofilm producers. Although the percentage positivity in pus samples was lower than that of vaginal and wound swabs, pus contributed the highest absolute number of biofilm-producing isolates. This finding suggests that biofilm formation may play an important role in suppurative and wound-related infections, where damaged tissue and inflammatory material can support bacterial adherence and survival.

Among device-associated and systemic specimens, urine samples showed a biofilm positivity rate of 59.38%, with 19 out of 32 isolates producing biofilm. This may be related to catheter-associated urinary tract infections, where bacteria can attach to catheter surfaces and persist despite antimicrobial therapy. Central line tips also showed biofilm production in 6 out of 13 isolates, indicating the importance of biofilm formation in intravascular device-related infections. In contrast, blood isolates showed a lower positivity rate of 36.00%, with 9 out of 25 isolates producing biofilm. This may be because bacteria isolated from blood are often in a disseminated or planktonic phase rather than an established surface-attached biofilm state. None of the miscellaneous swabs showed biofilm production.

The high rate of biofilm production in samples from mucosal surfaces, wounds, urine, and central line tips highlights the clinical importance of biofilm detection. Biofilm-producing organisms are more difficult to eradicate because the extracellular matrix protects them from antibiotics and host immune responses. Therefore, management of such infections may require not only appropriate antimicrobial therapy but also removal of infected devices, drainage of collections, or surgical debridement whenever indicated.[16,19,22]

Department-wise analysis also showed considerable variation in biofilm production. Overall, biofilm-producing isolates were detected across several hospital departments, with positivity ranging from 0% to 100%. The highest positivity was observed in ENT and NICU, where all 6 isolates from each department were biofilm producers. In the NICU, the use of invasive devices such as endotracheal tubes, umbilical catheters, and central lines may provide

surfaces for bacterial attachment. Similarly, ENT infections often involve mucosal surfaces where bacteria may persist as biofilm communities, contributing to chronic or recurrent infection.[19,23,24]

Obstetrics and Gynaecology also showed a high biofilm positivity rate of 76.92%, with 40 out of 52 isolates producing biofilm. This may be explained by the presence of mucosal surfaces, post-operative wounds, sutures, urinary catheters, and other foreign materials that favour bacterial attachment. Orthopaedics showed 50.00% positivity, while General Surgery showed 44.79% positivity. These findings are clinically relevant because surgical wounds, devitalised tissue, implants, and prosthetic materials can act as important sites for staphylococcal biofilm formation.[19,20]

Paediatrics, Dermatology, and OPD showed moderate biofilm positivity rates of 47.37%, 39.22%, and 33.33%, respectively. These departments mostly included superficial skin, soft-tissue, urine, or blood-related specimens, where biofilm formation may depend on the nature of infection, duration of illness, and presence or absence of medical devices. No biofilm-producing isolates were detected from General Medicine, Psychiatry, or PICU in this study. However, the number of isolates from these departments was small, so these findings should be interpreted carefully.

The high frequency of biofilm production in departments such as NICU, Obstetrics and Gynaecology, Orthopaedics, and General Surgery is important from a treatment and infection-control perspective. Infections caused by biofilm-forming staphylococci may respond poorly to routine antibiotic therapy alone. Proper source control, removal or replacement of infected devices, wound care, and selection of effective antimicrobial agents are essential for better clinical outcomes.[12,19,23]

In the subset analysis of *Staphylococcus aureus*, 56 out of 141 isolates were confirmed as biofilm producers, giving a positivity rate of 39.72%. Among these biofilm-producing *S. aureus* isolates, males accounted for the majority, with 50 out of 56 cases. This male predominance may be related to the larger number of pus and wound specimens from male patients, particularly from surgical and trauma-related infections. Since biofilm formation is mainly determined by bacterial characteristics rather than patient sex, this pattern is more likely due to differences in specimen source and clinical presentation.

Methicillin resistance was observed in 26 out of 56 biofilm-producing *S. aureus* isolates, accounting for 46.43%. The presence of both methicillin resistance and biofilm production is clinically significant, as it can make infections more difficult to treat. Biofilm formation limits antibiotic penetration and supports the survival of slow-growing or dormant bacterial cells, while methicillin resistance reduces the effectiveness of beta-lactam antibiotics. Together, these factors may contribute to persistence, recurrence, and treatment failure in staphylococcal infections.[14,19,20]

Overall, these findings emphasize the need for routine identification of biofilm-producing staphylococci, especially from device-associated infections, wounds, urine samples, and high-risk hospital units. Early recognition of biofilm formation can help clinicians choose appropriate treatment strategies and strengthen infection-control measures.

Department-wise and specimen-wise analysis of biofilm-producing *Staphylococcus aureus* showed that most isolates were recovered from invasive and surgical infections. Pus was the predominant specimen, accounting for 50 out of 56 biofilm-producing *S. aureus* isolates. These isolates were mainly obtained from General Surgery (n = 24), Dermatology (n = 14), ENT (n = 6), and Orthopaedics (n = 6). This finding suggests that biofilm formation may have an important role in wound infections, abscesses, and deep-seated soft-tissue infections. In such conditions, damaged tissue, necrotic material, and inflammatory exudates may provide favourable surfaces for bacterial attachment and persistence.

Urine samples accounted for 6 out of 56 biofilm-producing *S. aureus* isolates and were recovered exclusively from the General Surgery department. This may be related to postoperative catheter use, where bacteria can adhere to the catheter surface and form biofilm. The isolation of biofilm-forming *S. aureus*, particularly methicillin-resistant strains, from pus and catheter-associated specimens is clinically important. These infections may not respond adequately to beta-lactam antibiotics alone. Therefore, proper wound drainage, surgical debridement, catheter care or removal, and selection of suitable antimicrobial therapy are essential for effective management.[14,19]

Among the 159 coagulase-negative staphylococci isolates studied, 87 were confirmed as biofilm producers, giving a positivity rate of 54.72%. This higher rate supports the known role of CoNS as opportunistic pathogens, especially in device-associated and mucosal infections. CoNS have a strong ability to attach to artificial surfaces and damaged epithelial barriers, which allows them to persist in hospital settings and cause clinically significant infections.

In contrast to the male predominance observed among biofilm-producing *S. aureus*, biofilm-producing CoNS were more common in female patients. Of the 87 biofilm-positive CoNS isolates, 67 were from females and 20 were from males. This difference is likely related to the type of specimens and clinical conditions rather than a direct effect of sex. Female patients in this study were more frequently associated with obstetric, gynaecological, urogenital, and catheter-related specimens, where organisms such as *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* can readily adhere and form biofilms.[16,18]

Methicillin resistance was observed in 47 out of 87 biofilm-producing CoNS isolates, accounting for 54.02%. This finding is significant because CoNS can act as reservoirs of methicillin-resistance genes and may also produce extracellular slime or surface proteins that support biofilm formation. The combination of methicillin resistance and biofilm production can make these infections difficult to treat, as the biofilm matrix restricts antibiotic penetration while resistance mechanisms reduce the effectiveness of beta-lactam drugs.[11,16,23]

The department-wise distribution of biofilm-producing CoNS showed that Obstetrics and Gynaecology contributed the highest number of isolates, with 20 cases, including 14 wound swabs and 6 pus samples. This may be due to post-operative wounds, perineal infections, sutures, urinary catheters, and other local factors that support bacterial adherence. The NICU contributed 6 isolates, all recovered from central line tips, highlighting the importance of catheter-related biofilm formation in neonates, especially those requiring prolonged invasive support. General Surgery, OPD, and Paediatrics each contributed 7 biofilm-positive CoNS isolates, recovered from pus, urine, and blood specimens, respectively.

These findings show that CoNS should not always be dismissed as simple contaminants, particularly when isolated from devices, blood, wounds, or clinically relevant specimens. Their ability to form biofilm, along with frequent methicillin resistance, makes them important hospital-associated pathogens. Effective management should include careful clinical correlation, appropriate antimicrobial therapy, strict infection-control practices, and removal or replacement of infected devices whenever necessary.[11,23]

CONCLUSION

The present study showed that biofilm production was common among clinical staphylococcal isolates, with 143 out of 300 isolates showing biofilm-forming ability. Coagulase-negative staphylococci demonstrated a higher rate of biofilm production than *Staphylococcus aureus*, with positivity rates of 54.72% and 39.72%, respectively. This finding highlights the growing clinical importance of CoNS, particularly in infections associated with catheters, implants, wounds, and other medical devices.

The distribution of biofilm-producing isolates appeared to be influenced mainly by the site of infection, type of specimen, and exposure to medical devices. Biofilm-producing *S. aureus* isolates were more common in males, largely due to their association with pus samples, trauma-related wounds, and surgical infections. In contrast, biofilm-producing CoNS were more frequent in females, especially in specimens from obstetric, gynaecological, urogenital, and catheter-associated infections.

High biofilm positivity was observed in wound swabs, vaginal swabs, and isolates from high-risk units such as NICU and ENT. These findings suggest that damaged tissues, mucosal surfaces, and indwelling devices provide favourable conditions for bacterial attachment and persistence.

Biofilm production was also frequently associated with methicillin resistance, including MRSA and methicillin-resistant CoNS. This combination can make infections more difficult to treat, as biofilms reduce antibiotic penetration and help bacteria survive despite therapy. Therefore, routine detection of biofilm production, appropriate antibiotic selection, strict infection-control practices, timely device removal, and proper wound debridement are important for effective management of staphylococcal infections.

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