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Microbiological Detection and Characterization of Bacterial Biofilms Associated with Orthopedic Implant Infections

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ABSTRACT

Introduction:

Orthopedic implant infections are a significant type of surgical site infection, leading to high morbidity and mortality. The presence of implants in trauma surgeries increases the risk of microbial contamination, with many pathogens forming biofilms that enhance antibiotic resistance.

Objectives:

To identify the aerobic bacterial pathogens from pus samples of orthopedic implant site infections and evaluate their antibiotic susceptibility. Additionally, to assess the ability of these isolates to form biofilms.

Materials and Methods:

Pus samples from patients with suspected orthopedic implant infections were collected over 18 months and processed following CLSI guidelines. Biofilm formation was assessed using the Congo Red Agar (CRA) method&tube method.

Results:

Out of 75 pus samples, 46 (61.3%) showed bacterial growth, with 35 Gram-positive and 11 Gram-negative isolates. Staphylococcus aureus (30; 65.2%) was the most common Gram-positive pathogen, while CoNS accounted for 5 cases. Among Gram-negative isolates, Klebsiella spp. (5) was predominant, followed by E. coli and Pseudomonas spp. (3 each). Biofilm formation was observed in 50–100% of isolates across methods, with all Pseudomonas spp. and most S. aureus and Klebsiella spp. demonstrating biofilm-producing ability.

Conclusions:

Staphylococcus aureus (MSSA) is the predominant cause of orthopedic implant infections. Gram-negative isolates show multidrug resistance, and a large proportion of pathogens are biofilm producers. Antibiotic therapy should be guided by local susceptibility patterns.

KEYWORDS: CRA, Tube Method, Antibiotic sensitivity, Biofilm Formation, Orthopedic Implants

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INTRODUCTION

With advancements in orthopaedic care, prosthetic replacement and implant surgeries are now routinely performed and play a crucial role in alleviating pain and enhancing mobility in patients with joint damage. However, post-operative infection remains one of the most serious and challenging complications [1]. Early identification of infections following prosthetic replacement and implant surgery is often difficult, yet it is crucial for effective management and prevention of further complications. These infections contribute to extended hospital stays, elevated healthcare costs, increased morbidity and mortality, and a higher risk of readmission as well as the need for surgical debridement [2]. In India, the prevalence of infections related to orthopaedic

implants has been reported to be around 2.6%. Based on the timing of presentation, such infections are classified as early (within two weeks post-surgery), delayed (2–10 weeks), and late infections (beyond 10 weeks after surgery)[3].

Orthopedic implant site infection is primarily influenced by the severity of soft-tissue and periosteal damage following fracture, as devascularized bone and necrotic tissue favor bacterial growth and impair immune defense, leading to delayed fracture healing. Additional risk factors include comorbidities such as diabetes mellitus, rheumatoid arthritis, sickle cell anemia, malnutrition, obesity, immunosuppression, and existing infections elsewhere in the body (e.g., UTI).The source of infection may be endogenous or exogenous. Patients may acquire infection from themselves or through cross-transmission from other patients or healthcare personnel, as well as from environmental sources such as air, water, food, medications, medical equipment or instruments, linen, and hospital waste during the postoperative period[4-6].

The pathogens responsible for these infections may originate from either endogenous or exogenous sources; however, the majority arise from the patient's own endogenous bacterial flora under favourable conditions [7,8]. The most commonly implicated microorganisms include Staphylococcus aureus, coagulase-negative Staphylococcus spp. (CONS), Enterococcus spp., Escherichia coli, Klebsiella spp., Proteus mirabilis, and Pseudomonas spp. [9]. Implant-associated infections typically occur due to bacterial adhesion to the implant surface, followed by biofilm formation at the site of implantation. Once established, biofilms are difficult to eradicate by host immune defenses and conventional antimicrobial therapy [10].

Currently, many bacterial isolates exhibit resistance to commonly used first-line antibiotics. Moreover, the microbial flora and antibiotic susceptibility patterns vary from one hospital to another, making empirical antibiotic therapy challenging. In view of this, the present study was undertaken to assess the prevalence of causative organisms, their antimicrobial susceptibility profiles, and their capacity to form biofilms on implants used in orthopaedic surgeries.

MATERIAL & METHDOLOGY

The present study was carried out in the Department of Microbiology at Integral Institute of Medical Sciences and Research, Lucknow, during the period from March 2023 to 2025. A total of 75 patients who had undergone orthopaedic implant or prosthetic surgery and subsequently presented with clinical features suggestive of infection were included in the study. Prior to enrolment, written informed consent was obtained from all participants, and approval was granted by the Institutional Ethics Committee. Patient details were documented using a structured proforma, which included demographic information and relevant clinical variables such as age, sex, diagnosis, associated comorbidities, smoking history, nutritional status, and the type of implant used.

Specimens for bacteriological analysis were collected from discharge surrounding the infected implant and adjacent tissues using sterile cotton swabs or sterile disposable syringes, as appropriate. All samples were promptly transported to the microbiology laboratory in suitable sterile containers according to the nature of the specimen.

Each specimen was subjected to Gram staining, and acid-fast staining was performed for aspirated samples when indicated. Aerobic bacterial culture was carried out by inoculating specimens onto Blood agar, MacConkey agar, and thioglycollate medium, followed by incubation at 37°C for 24 hours.

Bacterial isolates were identified using standard biochemical methods. Antimicrobial susceptibility testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines 2024, employing the Kirby–Bauer disk diffusion technique[8].

Biofilm formation by the isolates was assessed using two phenotypic methods: the Congo Red Agar (CRA) method (Figure 1) and the Tube method (Figure 2), performed according to the protocol described by Afreenish Hassan et al.[9]

RESULTS

A total of 75 pus samples collected from orthopedic implant sites were processed in the Microbiology Laboratory for culture and antimicrobial susceptibility testing. Significant bacterial growth was observed in 46 (61.3%) samples, while the remaining samples showed no growth. Among the culture-positive specimens, gram-positive cocci (GPC) were isolated in 35 cases, whereas gram-negative bacilli (GNB) were recovered from 11 cases (Figure 3).

Klebsiella species were the most commonly isolated gram-negative organisms, accounting for 5 isolates, followed by Escherichia coli and Pseudomonas species, with 3 isolates each. Among the gram-positive organisms, Staphylococcus aureus was the predominant pathogen, identified in 30 cases, while coagulase-negative Staphylococcus (CoNS) was isolated in 5 cases (Figure 4).

EMERGINGANTIMICROBIAL RESISTANCE IN IMPLANT-ASSOCIATED INFECTIONS

Among the 46 culture-positive pus samples, Staphylococcus aureus was the most common isolate (30/46; 65.2%), with 16/30 (53.3%) identified as MRSA; all MRSA isolates were biofilm producers. S. aureus showed high resistance to ciprofloxacin (28/30; 93.3%) and levofloxacin (27/30; 90%), along with considerable resistance to gentamicin, doxycycline, tetracycline, erythromycin, and clindamycin, while vancomycin and teicoplanin remained largely effective and linezolid showed 100% susceptibility (30/30). Coagulase-negative staphylococci (CoNS) accounted for 5/46 (10.9%) isolates, of which 3/5 (60%) were methicillin-resistant; all CoNS isolates were resistant to ciprofloxacin (5/5; 100%) and most to levofloxacin (4/5; 80%),

whereas vancomycin and linezolid retained complete activity (5/5). Pseudomonas aeruginosa was isolated in 3/46 (6.5%) samples and demonstrated multidrug resistance, with 100% resistance (3/3) to levofloxacin, ceftazidime, cefepime, tobramycin, and piperacillin, but complete susceptibility to polymyxin B and colistin. Klebsiella species (5/46; 10.9%) showed high resistance to fluoroquinolones (ciprofloxacin 5/5; 100%, levofloxacin 4/5; 80%) and several β-lactams, while amikacin and tigecycline remained the most effective agents. Escherichia coli was isolated in 3/46 (6.5%) cases and exhibited multidrug resistance, including 100% resistance to cefazolin, with better susceptibility to carbapenems, ceftazidime–clavulanic acid, and tigecycline (Table 3).

BIOFILM PRODUCTION AMONG BACTERIAL ISOLATES

Of the 46 culture-positive orthopedic pus samples, 35 isolates were Gram-positive, including 30 Staphylococcus aureus and 5 coagulase-negative staphylococci (CoNS). Biofilm formation in S. aureus was detected in 15 isolates by the Congo Red Agar (CRA) method and in 16 isolates by the tube method, while among CoNS, 2 isolates were positive by CRA and 3 by the tube method.

Among the 11 Gram-negative isolates, biofilm production was observed in 2 of 3 Escherichia coli isolates using both CRA and tube methods. Of the 5 Klebsiella spp. isolates, 3 showed biofilm formation by CRA and all 5 were positive by the tube method. All 3 Pseudomonas spp. isolates demonstrated biofilm production and were detected by both methods (Table 2).

GENDER WISE DISTRIBUTION

Of the 46 culture-positive samples, 33 were obtained from male patients and 13 from female patients, indicating a higher prevalence of culture positivity among males.

AGE DISTRIBUTION OF PATIENTS

The highest number of cases was observed in the 10–20 and 30–40year age groups, together accounting for 16 cases (34.8%). This was followed by the 20–30year age group with 9 cases (19.6%). Overall, adolescents and young adults constituted the majority of cases in the study (Table 1).

TIMING OF ONSET OF IMPLANT-ASSOCIATED INFECTIONS

Wound infections were categorized according to Trampuz and Zimmerli's classification[10]. Among the 46 culture-positive cases, 25 (54.3%) presented within 2 weeks of surgery (early), 12 (26.1%) between 2 and 10 weeks (delayed), and 9 (19.6%) after 10 weeks (late) postoperatively.

DISTRIBUTION OF SURGICAL WOUND CLASSIFICATION AMONG STUDY PARTICIPANTS

Out of the 46 patients included in the study, 29 (63.04%) had clean wounds. Clean-contaminated wounds were observed in 8 patients (17.39%), while 7 patients (15.22%) had contaminated wounds. Only 2 patients (4.35%) presented with dirty or infected wounds (Figure 5).

DISCUSSION

Orthopedic implant-related infections continue to pose significant diagnostic and therapeutic challenges. The presence of bacterial biofilms further complicates management, contributing to limited antibiotic options and necessitating prolonged antimicrobial therapy due to the emergence of multidrug-resistant organisms. Accurate sample collection is critical for reliable microbiological diagnosis, as it directly impacts culture yield. Standard methods for obtaining samples include direct swabbing of the site, aspiration of periprosthetic fluid, and retrieval of the implant followed by sonication. Even with significant progress in antimicrobial treatments, infections at orthopedic implant sites remain a leading cause of treatment failure and patient morbidity. Infections associated with implants still present a significant challenge for orthopedic surgeons.Esteban and colleagues reported that sonication increased culture sensitivity from 84.2% to 94.7% compared with conventional periprosthetic tissue cultures. In contrast, Gomez et al.,[12]observed a lower culture positivity rate of 60%. Higher detection rates of 89% and 93.9% were reported by Zimmerli et al. and Khosravi et al., respectively. In the present study, culture positivity was observed in 61.3% of the samples analyzed, which is comparable with results reported in the literature and supports the usefulness of optimized sampling techniques for detecting implant-associated infections.

Our study revealed that Staphylococcus aureus was the predominant organism responsible for orthopedic implant site infections, a finding consistent with previous reports by Anisha Fernandez et al.,[2]Khosravi et al.,[4] Singh Nidhi et al.,[14] and Vishwajith et al[1].

The second most frequently isolated organisms in our study were coagulase-negative staphylococci (CONS) and Escherichia coli. CONS, being a normal skin commensal, may have been introduced to the surgical site due to inadequate skin disinfection during surgery or potentially from improper handling during sample collection. The distribution of organisms observed suggests the involvement of nosocomial pathogens present in the operating theater or post-operative wards, where patients undergo regular monitoring and dressing changes. A limitation of this study was the exclusion of anaerobic cultures, which can also contribute to implant site infections, particularly those occurring more than 24 months after surgery[4]; however, none of the patients in this study presented beyond that timeframe. Additionally, the recent use of antibiotics by most patients may have

further reduced the likelihood of isolating anaerobic organisms [13].

The antimicrobial susceptibility pattern showed a high level of multidrug resistance among Gram-negative isolates, with susceptibility largely limited to amikacin, imipenem, and meropenem, while routine prophylactic cephalosporins were ineffective due to widespread resistance. Amikacin may be used in patients with normal renal function, whereas imipenem is preferable in those with renal compromise. In contrast, most Gram-positive cocci, including Staphylococcus aureus and coagulase-negative staphylococci, remained methicillin sensitive, supporting the use of cloxacillin over higher-end antibiotics. Hence, empirical therapy with imipenem and cloxacillin may be considered for suspected orthopedic implant–associated infections until susceptibility results are available.

In the present study, early infection was observed in 25 patients (54.34%), a finding comparable to that reported by Khosravi et al.,[4] who noted early infections in 72.9% of cases. This pattern suggests that most implant-associated infections are likely acquired at the time of surgery and are commonly caused by organisms of relatively low virulence.

In the present study, biofilm formation was assessed using three methods: Congo Red Agar (CRA),and Tube Method (TM). Staphylococcus aureus emerged as the predominant biofilm-producing organism, likely reflecting its higher frequency of isolation in culture.The ability of these organisms to produce biofilms may account for the prolonged duration of antimicrobial therapy and extended hospital stays observed in our patients, thereby contributing to increased morbidity.

CONCLUSION

Orthopedic implant–associated infections remain a significant diagnostic and therapeutic challenge, contributing substantially to patient morbidity. The findings of this study highlight the importance of meticulous evaluation of predisposing risk factors, wound classification, and appropriate perioperative antibiotic prophylaxis to effectively prevent implant site infections. Staphylococcus aureus, particularly methicillin-sensitive strains, emerged as the predominant causative organism, underscoring the need for institution-specific antimicrobial susceptibility data to guide rational antibiotic therapy. The frequent ability of isolates to form biofilms further complicates management, as it contributes to persistent infection, prolonged treatment, and extended hospital stay. Strict adherence to universal infection control measures, especially hand hygiene in operation theatres and postoperative wards, is essential to reduce infection rates. Further large-scale studies with extended follow-up are warranted to better understand the epidemiology, resistance patterns, and clinical outcomes associated with orthopedic implant–related infections.

Figure 1: Congo Red Agar Method

Figure 2: Tube Method

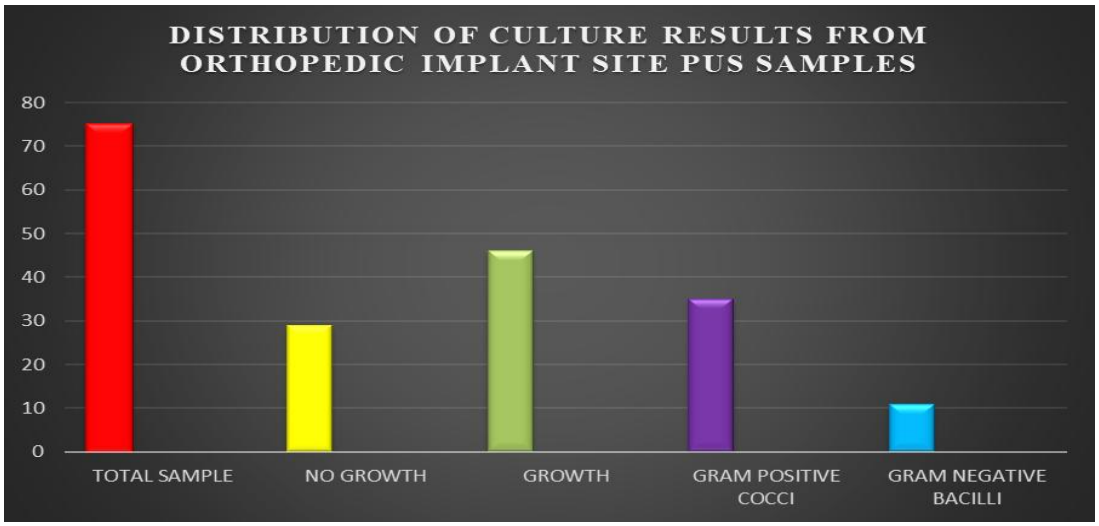

Figure 3: Distribution of culture results from Orthopedic implant site pus samples

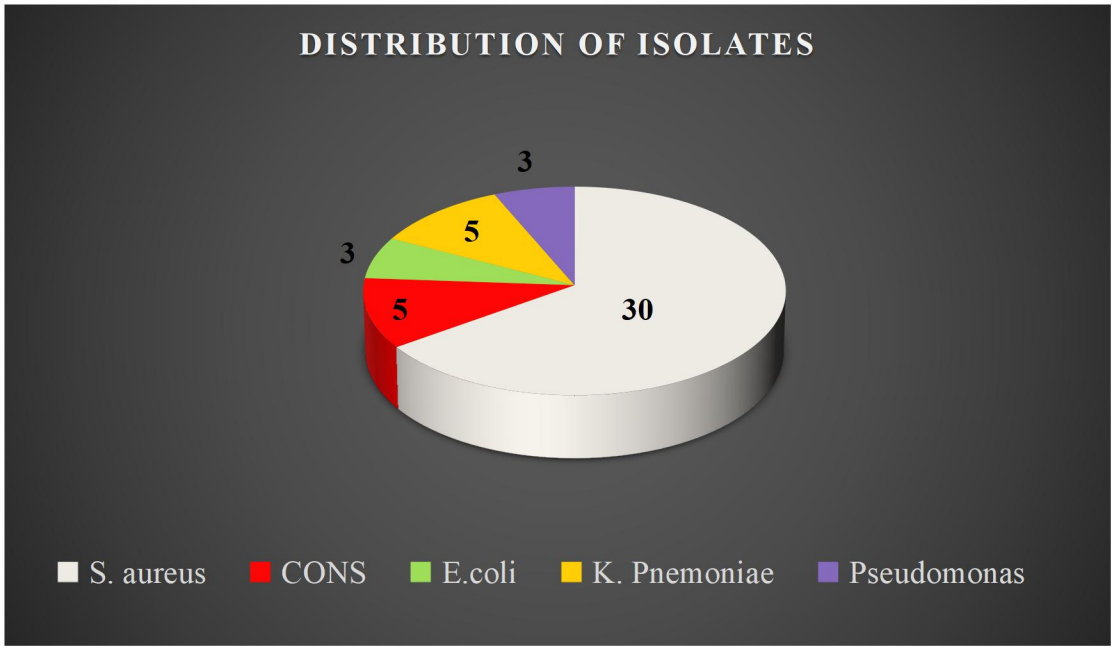

Figure 4: Distribution of isolates

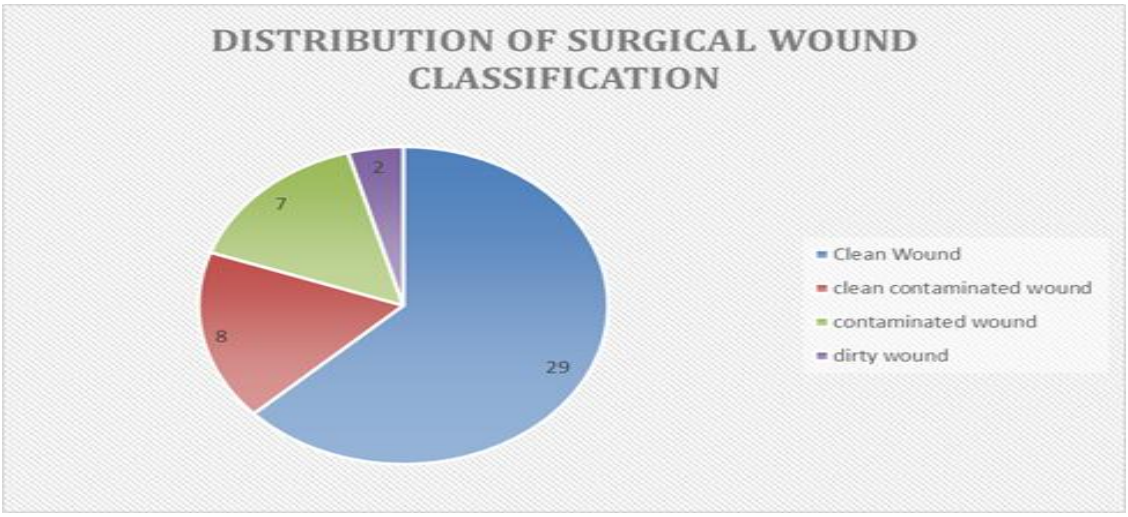

Figure 5: Distribution of Surgical wound classification

TABLE: 1 DISTRIBUTION OF AGE & GENDER		
AGE GROUP	**MALE**	**FEMALE**
0-10	2	00
10-20	8	02
20-30	7	02
30-40	8	04
40-50	3	01
50-60	3	01
60-70	2	03

TABLE: 2 BIOFILM PRODUCTION AMONG CLINICAL ISOLATES USING CRA AND TUBE METHODS			
ORGANISM	**TOTAL ISOLATES**	**POSITIVE FOR BIOFILM (TM)**	**POSITIVE FOR BIOFILM (CRA)**
GRAM POSITIVE			
Staphylococcus	30	16	15
CONS	5	03	02
GRAM NEGATIVE			
E. coli	3	02	02
Klebsiella spp.	5	05	03
Pseudomonas spp.	3	03	03
TOTAL	46	29	25

Table:3 Consolidated Antibiotic Resistance Pattern of Bacterial Isolates					
Antibiotic	***S. aureus*** **(n=30)**	**CoNS (n=5)**	***E. coli*** **(n=3)**	***Klebsiella*** **spp. (n=5)**	***P. aeruginosa*** **(n=3)**
Cefoxitin	16	3	—	—	—
Erythromycin	12	3	—	—	—
Clindamycin	10	2	—	—	—
Tetracycline	12	1	1	3	—
Doxycycline	13	1	—	—	—
Vancomycin	2	0	—	—	—
Teicoplanin	3	1	—	—	—
Linezolid	0	0	—	—	—
Ciprofloxacin	28	5	2	5	1
Levofloxacin	27	4	2	4	3
Gentamicin	13	3	1	2	2
Tobramycin	6	2	1	2	3
Ampicillin–Sulbactam	—	—	2	3	—

Piperacillin–Tazobactam	—	—	2	2	2
Piperacillin	—	—	—	—	3
Ticarcillin–Clavulanic acid	—	—	—	—	2
Ceftriaxone	—	—	2	3	—
Ceftazidime	—	—	2	3	3
Ceftazidime–Clavulanic acid	—	—	1	2	1
Cefepime	—	—	2	2	3
Aztreonam	—	—	2	3	2
Imipenem	—	—	1	3	2
Meropenem	—	—	1	2	2
Doripenem	—	—	1	3	2
Tigecycline	—	—	1	1	—
Cefazolin	—	—	3	—	—
Polymyxin B	—	—	—	—	0
Colistin	—	—	—	—	0

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