

**Original Research Article** 

# STUDY OF BIOFILM FORMATION IN PATIENTS UNDERGOING ORTHOPAEDIC IMPLANT SURGERY IN RAJASTHAN

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 Received
 : 03/02/2025

 Received in revised form : 05/04/2025

 Accepted
 : 22/04/2025

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DOI: 10.70034/ijmedph.2025.2.394

Source of Support: Nil, Conflict of Interest: None declared

**Int J Med Pub Health** 2025; 15 (2); 2189-2192

#### ABSTRACT

**Background:** In implant-associated infections, organisms attach to the surface of the implant, and certain isolates may interact with host proteins to produce biofilms resulting in the persistence of infections. Aim of this investigation was to study biofilm formation among patients undergoing orthopaedic implant surgery.

**Materials and Methods:** A total of 100 samples of infections following orthopaedic implant surgery were included. Samples were subjected to gram staining, acid-fast bacilli staining, 10% KOH preparation and antibiotic sensitivity.

**Results:** Growth was observed in 80 (80%) samples. The majority of isolates from orthopedic implant infections are Pseudomonas aeruginosa 37 (46.25%), Staphylococcus aureus 25 (31.25%), E. coli 13 (16.25%) & Klebsiella in 05 (6.25%) samples. biofilm was observed in 92 out of 100 cases of implant removal surgery. Pseudomonas aeruginosa exhibits complete sensitivity to Gentamycin, Imipenem, and Levofloxacin. Klebsiella pneumonia exhibits complete sensitivity to Levofloxacin, Cefuroxime, and Piperacillin+Tazobactam, while showing 75% sensitivity to Cefepime and Imipenem.

**Conclusion:** This study reinforces the critical role of biofilm-forming pathogens, particularly Pseudomonas aeruginosa and Staphylococcus aureus, in orthopaedic implant infections.

Keywords: Biofilm, Implant-Related Infection, Antibiotics sensitivity, Implant, Pathogens.

# **INTRODUCTION**

A growing number of individuals worldwide are experiencing bone and joint issues either from ageing or injuries. Elective and emergency orthopaedic procedures entail incising, drilling, or manipulating the bone for joint replacement or realignment of long bonesImplant surgery is prevalent in orthopaedic procedures for effectively reducing pain and enhancing mobility in compromised joints.<sup>[1]</sup> Infections related to fracture fixation may arise exogenously in instances of open trauma, during the implantation of the fixation device, or due to impaired wound healing.<sup>[2]</sup> Stainless steel implants exhibit markedly higher infection rates compared to titanium implants.<sup>[3]</sup> A soft tissue response to steel

implants, results in the development of a fibrous capsule that encases a liquid-filled cavity.<sup>[4,5]</sup> Bacteria can proliferate and disseminate unrestricted in this avascular region, which is also less accessible to host defence mechanisms. Preventing initial bacterial attachment is crucial, as established biofilms are exceedingly challenging to manage. In implant-associated infections, organisms attach to the surface of the implant, and certain isolates may interact with host proteins to produce biofilms. This results in the persistence of infections and occasionally in the emergence of resistance to antimicrobial treatment. The predominant organisms isolated in implant infections are Staphylococcus aureus and other gram-negative bacilli. These organisms possess the capability for biofilm production. Infections are severe problems following such surgery.<sup>[6]</sup>

Over the past century, the prevalence of infection has significantly diminished owing to modern surgical facilities and aseptic protocols. Nevertheless, they continue to provide a challenge in developing countries, characterised by rising morbidity rates and significant expenses. Aggressive treatment interventions, including extended courses of potent antibiotics, supplementary surgeries, and extended rehabilitation, are linked to problems that necessitate extended hospitalisation and may result in repeated impairment. The healthcare expenses associated with revision procedures are substantial, and the risk of infection is elevated compared to primary surgeries, so imposing a strain on both patients and healthcare facilities. Aim of this investigation was to study biofilm formation among patients undergoing orthopaedic implant surgery in Rajasthan state.

# **MATERIALS AND METHODS**

The research was performed in the Department of Orthopaedics at Pacific Medical College and Hospital, Udaipur, Rajasthan during March 2024 to February 2025. Department of Microbiology of a tertiary care teaching centre from Etah district of Uttar Pradesh provided us intellectual inputs to carry out this study. A total of 100 samples of infections following orthopaedic implant surgery were included. Purposive sampling technique was adopted. **Sample collection technique** 

Samples collection technique followed standard procedures. Aseptic conditions were maintained. Infected tissue and pus samples were collected in a sterile vial from the interface of the plate and bone, subsequently mixed with normal saline as shown in [Figure 1].



Figure 1: Collected sample in a sterile vial

**Site of availability of Biofilm:** Biofilm adhering to the outer surface of bone and the inner surface of the plate, as well as biofilm adhering to the screw hole in the nail, was extracted with strict aseptic measures and placed in a sterile vial mixed with normal saline. Figure 2 shows formation of biofilm at screw hole at interlocking nail as site of availability of biofilm. [Figure 2]



Figure 2: Formation of biofilm at screw hole at interlocking nail

**Sample processing technique:** The sample was inoculated into Nutrient agar, Blood agar, and MacConkey agar plates and incubated at 37 degrees Celsius for 24 hours. Subsequent to the processing of such sample for four important aspects

- a) Gram staining
- b) Acid-fast bacilli staining
- c) 10% KOH Preparation
- d) Antibiotic Sensitivity

Data regarding gram staining, acid-fast bacilli staining, KOH preparation and antibiotic sensitivity along with all the relevant details pertaining to this study was captured in a self-designed proforma. Collected data was entered in the MS Excel spreadsheet, coded appropriately and later cleaned for any possible errors. Analysis was carried out using SPSS (Statistical Package for Social Sciences) for Windows version 22.0. Categorical data was presented as frequency and percentages.

# **RESULTS**

The mean age of participants in the study was  $33.8\pm8.5$  years, with a range of 11 to 58 years. The predominant age group in my study was 25-45 years, including around 46% of the total individuals, while the second most prevalent group was 46-65 years, representing 32% of the total subjects. In our study of 100 people, males (n=72, 72%), outnumbered female participants. Of total 100 patients, 46 (46%)

individuals exhibited no risk factors. 25 (25%) were smoked tobacco consumers; 15 (15%) had a history of chewing form of tobacco consumption.

In a study of 100 subjects, 70 (70%) had a closed fracture pattern, while 30 (30%) presented with an open grade fracture pattern. In a study involving 100 subjects, fracture repair techniques employed included closed reduction in 48 cases (48%), open reduction in 46 cases (46%), and MIPPO method in 6 cases (6%).

Of total 100 study individuals, 39 (39%) had interlocking nail implants, while 33 (33%) had plates removed during surgery. In our analysis, the femur was the most frequently infected bone in the lower limb, accounting for 40 cases (40%), whereas the bones of the forearm were the most usually infected in the upper limb, including 9 cases (9%).

Out of total 100 study subjects, stainless steel implants were utilised in 88 (88%) instances, whilst titanium implants were employed in 12 (12%) instances. In a cohort of 100 study individuals, early, delayed, and late onset infections were observed in 40 (40%), 29 (29%), and 31 (31%) patients, respectively. Results of study reveals that growth was observed in 80 (80%) samples. The majority of isolates from orthopedic implant infections are Pseudomonas aeruginosa 37 (46.25%), Staphylococcus aureus 25 (31.25%), E. coli 13 (16.25%) & Klebsiella in 05 (6.25%) samples. [Table 1].

Table 1: Growth status and bacteriological profile of study samples.					
Status	Organism	N (%)			
Growth (n=80)	Pseudomonas aeruginosa	37 (46.25%)			
	Staphylococcus aureus	25 (31.25%)			
	E. Coli	13 (16.25%)			
	Klebsiella	05 (6.25%)			
No Growth		20 (20%)			

In our study, biofilm was observed in 92 out of 100 cases of implant removal surgery. In a study involving 100 subjects undergoing implant removal, 90 individuals (90%) achieved union at the fracture site, while 10 individuals (10%) experienced non-union at the fracture site.

Our findings indicate that Pseudomonas aeruginosa exhibits complete sensitivity to Gentamycin, Imipenem, and Levofloxacin. Additionally, sensitivity to Piperacillin+Tazobactam is observed at 84%, while ceftazidime shows a sensitivity rate of 50%. Klebsiella pneumonia exhibits complete sensitivity to Levofloxacin, Cefuroxime, and Piperacillin+Tazobactam, while showing 75% sensitivity to Cefepime and Imipenem. Escherichia coli exhibit complete sensitivity to cefuroxime, with an 80% sensitivity observed for Levofloxacin, Imipenem, and Piperacillin+Tazobactam. Staphylococcus aureus exhibits complete sensitivity to Amoxyclav, Vancomycin, Gentamycin, and Doxycycline, while showing a sensitivity rate of 57% to Azithromycin and Clindamycin.

Table 2: Antibiotics Sensitivity pattern among study samples.									
Antibiotic disc	P. aeruginosa (n=37)		S. aureus (n=25)		E. Coli (n=13)		Klebsiella (n=5)		
	Ν	%	Ν	%	Ν	%	Ν	%	
Gentamycin	37	100	25	100	-	-	-	-	
Levofloxacin	35	94.6	-	-	10	76.9	5	100	
Cefepime	30	81.1	-	-	8	61.5	4	80	
Cefuroxime	22	59.5	-	-	13	100	5	100	
Imipenam	37	100	-	-	9	69.2	3	60	
Piperacillin+Tazobactam	28	75.7	-	-	10	76.9	4	80	
Vancomycin	-	-	25	100	-	-	-	-	
Doxycyclin	-	-	24	96	7	53.8	-	-	
Clindamycin	-	-	13	52	-	-	-	-	
Azithromycin	-	-	12	48	-	-	-	-	

# DISCUSSION

In the realm of orthopaedic implant infections, the predominant organisms identified through standard microbiological protocols are Staphylococcus aureus and Staphylococcus epidermidis. These organisms have the ability to generate biofilms. The present study on biofilm formation in patients undergoing orthopaedic implant surgery in Rajasthan provides critical insights into the microbiological landscape and antibiotic sensitivity patterns associated with implant-related infections. These findings have significant implications for clinical management and infection control strategies in orthopaedic practice. Our results demonstrate a high prevalence of biofilm formation, observed in 92% of implant removal cases, underscoring the pervasive role of biofilms in implant-associated infections. This aligns with previous studies highlighting biofilms as a major contributor to the persistence and chronicity of orthopaedic implant infections, complicating treatment outcomes.<sup>[7-9]</sup> The predominance of Pseudomonas aeruginosa (46.25%) and Staphylococcus aureus (31.25%) as the principal pathogens corroborates findings from other regional and international studies, which identify these organisms as key biofilm producers in implant infections.<sup>[10]</sup> The isolation of gram-negative bacilli

such as Escherichia coli and Klebsiella spp. further reflects the polymicrobial nature of these infections and their potential for antimicrobial resistance.<sup>[9]</sup>

The predominance of stainless steel implants (88%) over titanium (12%) in our cohort, with a corresponding higher infection rate, supports existing literature indicating that stainless steel implants are more susceptible to infection than titanium, likely due to the formation of a fibrous capsule around steel implants that creates an avascular niche conducive to bacterial colonization and biofilm development.<sup>[10]</sup> This biological environment limits host immune access and facilitates bacterial proliferation, emphasizing the need for careful implant material selection and surgical technique optimization.<sup>[11]</sup>

Antibiotic sensitivity patterns revealed that Pseudomonas aeruginosa isolates retained full sensitivity to Gentamycin, Imipenem, and Levofloxacin, while showing reduced sensitivity to ceftazidime (50%). Staphylococcus aureus isolates were fully sensitive to Amoxyclav, Vancomycin, Gentamycin, and Doxycycline but exhibited partial resistance to Azithromycin and Clindamycin. These findings highlight the complexity of managing biofilm-associated infections, where standard antibiotic regimens may be insufficient due to biofilm-mediated resistance mechanisms. The observed sensitivity to broad-spectrum agents such as Imipenem and Piperacillin-Tazobactam suggests their utility in empirical therapy; however, the potential for resistance development necessitates judicious use guided by culture and sensitivity results.[12]

The demographic profile, with a majority of patients aged 25-45 years and a male predominance, reflects the active population segment most likely to sustain trauma requiring orthopaedic implants. The predominance of closed fracture patterns (70%) and the femur as the most frequently infected bone (40%) are consistent with the anatomical and clinical patterns of injury and implant use in this region. The presence of risk factors such as tobacco use in 40% of patients may contribute to impaired wound healing and susceptibility to infection, warranting targeted perioperative counseling and intervention.<sup>[13,14]</sup>

The clinical implications of these findings are profound. The high incidence of biofilm formation necessitates the development of strategies to prevent initial bacterial adhesion, including the use of implant coatings, improved surgical asepsis, and perioperative antibiotic prophylaxis tailored to local microbiological profiles. Furthermore, the management of established infections requires a multidisciplinary approach combining surgical debridement, implant removal when necessary, and prolonged, targeted antimicrobial therapy.<sup>[15]</sup>

Limitations of this study include its single-center design and purposive sampling, which may limit generalizability. Future research should focus on longitudinal outcomes, molecular characterization of biofilm-forming strains, and evaluation of novel anti-biofilm agents.

### CONCLUSION

This study reinforces the critical role of biofilmforming pathogens, particularly Pseudomonas aeruginosa and Staphylococcus aureus, in orthopaedic implant infections in Rajasthan. The predominance of stainless steel implants as a risk factor and the complex antibiotic sensitivity patterns observed underscore the need for vigilant infection control measures and personalized antimicrobial strategies to improve patient outcomes and reduce the burden of implant-related infections.

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