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Predictors of Implant Failure in Orthopaedic Infections Due to Coagulase-Negative Staphylococci: The Impact of Biofilm and Proteolytic Enzymes – A Prospective Observational Study.

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Abstract

Background: Coagulase-negative staphylococci (CoNS), long considered low-virulence skin commensals, have emerged as significant pathogens in orthopaedic implant-associated infections. Their ability to form biofilms and produce proteolytic enzymes contributes to chronic infection, antimicrobial resistance, and implant failure.

Objectives: To evaluate the microbiological and biochemical characteristics of CoNS isolates from orthopaedic implant infections, assess antimicrobial susceptibility, and identify predictors of adverse clinical outcomes, including implant removal and delayed bone healing.

Methods: This prospective observational study enrolled 120 patients with suspected implant-associated infections over two years. Sonication fluid and periprosthetic tissue cultures were performed, and isolates underwent species identification, antimicrobial susceptibility testing, and assessment of biofilm formation, exopolysaccharide production, and protease activity. Clinical outcomes were recorded over a six-month follow-up. Statistical analyses included multivariate logistic regression and correlation studies.

Results: CoNS were isolated in 48 cases (40%), predominantly *Staphylococcus epidermidis* (70.8%). Methicillin resistance was present in 68.7% of isolates, with high rates of multidrug resistance to erythromycin (79.1%), ciprofloxacin (64.5%), and clindamycin (60.4%). Strong biofilm production was observed in 79.1% of isolates and was significantly associated with implant removal (73.6% vs. 30%; p=0.004), prolonged antibiotic therapy (mean 6.4 vs. 4.2 weeks; p=0.001), and delayed union (44.7% vs. 20%; p=0.03). Multivariate analysis identified strong biofilm production (OR 4.25; p=0.015) and higher proteolytic enzyme activity (OR 1.92; p=0.040) as independent predictors of implant removal.

Conclusions: CoNS are major contributors to orthopaedic implant failure, primarily driven by biofilm-related virulence and proteolytic activity rather than methicillin resistance alone. These findings highlight the need for early microbiological diagnosis and biofilm-targeted interventions.

Recommendations: Clinicians should incorporate implant sonication and biofilm assessment into routine diagnostic protocols and consider prolonged antimicrobial therapy and early surgical intervention in cases with strong biofilm-producing CoNS. Future research should focus on molecular characterization of virulence factors and anti-biofilm therapies to improve treatment outcomes

Keywords: *Coagulase-negative staphylococci, biofilm, orthopaedic implants, methicillin resistance, proteolytic enzymes, implant failure, antimicrobial resistance.*
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Introduction

Orthopaedic implants, such as plates, screws, intramedullary nails, and joint prostheses, have transformed the management of fractures and degenerative joint disease by enabling early mobilization and improved functional outcomes. Despite advances in surgical technique and perioperative care, implant-related infections remain one of the most feared complications, with reported incidences ranging from 1% to 5% in clean elective orthopaedic procedures and up to 30% in open fractures [1]. These infections often lead to prolonged hospitalization, repeated surgeries, impaired bone healing, and, ultimately, implant failure.

While *Staphylococcus aureus* has historically been the most common pathogen in implant-associated infections, coagulase-negative staphylococci (CoNS) have emerged as equally important, particularly in late-onset and chronic infections. CoNS are a heterogeneous group of Gram-positive cocci, widely regarded as commensals of the skin and mucosal surfaces. However, their increasing role as opportunistic pathogens in healthcare-associated infections underscores their clinical relevance [2]. Among the numerous CoNS species, *Staphylococcus epidermidis* is the most frequently isolated from orthopaedic implant infections, followed by *S. haemolyticus* and *S. lugdunensis* [3].

The pathogenicity of CoNS is primarily attributed to their exceptional ability to adhere to biomaterial surfaces and produce biofilms—a structured community of bacterial cells embedded in an extracellular matrix rich in exopolysaccharides (such as polysaccharide intercellular adhesin), teichoic acids, proteins, and extracellular DNA [4]. This biofilm matrix not only anchors bacteria firmly to implant surfaces but also serves as a protective barrier against host immune responses and antibiotics, rendering infections difficult to eradicate. Biofilm-associated cells exhibit up to 1,000-fold higher resistance to antimicrobials compared to planktonic cells [5]. Furthermore, CoNS produce a variety of biochemical factors, including proteolytic enzymes and phenol-soluble modulins, that contribute to tissue damage, immune evasion, and persistent infection [4].

Another critical concern is the high prevalence of antimicrobial resistance among CoNS isolates, particularly methicillin resistance mediated by the mecA gene encoding the altered penicillin-binding protein PBP2a. Methicillin-resistant CoNS (MR-CoNS) are frequently resistant to multiple classes of antibiotics, including macrolides, lincosamides, aminoglycosides, and fluoroquinolones, which severely limit therapeutic options [6-8]. The rising burden of multidrug-resistant strains complicates empirical treatment, increases treatment costs, and often necessitates prolonged administration of glycopeptides or oxazolidinones [6-8].

From a diagnostic perspective, distinguishing true CoNS pathogens from contamination remains a major challenge, especially since CoNS are ubiquitous contaminants in clinical microbiology laboratories. Conventional tissue cultures may yield false-negative results due to the biofilm-associated low metabolic activity of organisms. Innovative methods, such as implant sonication, have significantly improved diagnostic sensitivity by dislodging biofilm-embedded bacteria, while molecular techniques allow rapid species identification and resistance gene detection [9, 10].

Despite increasing recognition of their pathogenic role, comprehensive studies evaluating the microbiological and biochemical profiles of CoNS and their clinical impact on orthopaedic implant outcomes remain limited. This study was undertaken to characterize CoNS isolates in implant-associated orthopaedic infections, assess their biofilm-forming potential, quantify biochemical virulence factors such as exopolysaccharide production and proteolytic enzyme activity, determine antimicrobial resistance patterns, and correlate these findings with clinical outcomes including implant survival, delayed union, and need for revision procedures. Understanding these factors is essential for developing effective prevention strategies, optimizing treatment protocols, and improving patient care in orthopaedic implant surgery.

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Materials and Methods

Study Design and Setting

This was a prospective observational cohort study conducted over 24 months (January 2023–December 2024) in the Departments of Orthopaedics and Microbiology at Mahatma Gandhi Memorial (MGM) Hospital, Warangal, Telangana, India. MGM Hospital is a large tertiary care teaching institution affiliated with Kakatiya Medical College, serving as a referral center for trauma and orthopaedic cases across northern Telangana. The hospital is equipped with dedicated orthopaedic surgical units, microbiology laboratories with automated identification systems, and intensive rehabilitation services, providing a comprehensive setting for both clinical care and research.

The study protocol received approval from the Institutional Ethics Committee (Approval No.: KIEC/ORTHO/2023/067). Written informed consent was obtained from all participating patients.

Study Population

Patients of any age presenting with suspected implant-associated orthopaedic infections were eligible for inclusion. Infection was suspected in cases demonstrating persistent wound discharge beyond two weeks postoperatively, localized signs of inflammation (erythema, swelling, or tenderness) over the implant site, radiographic evidence of peri-implant osteolysis, or delayed fracture union suggestive of infection.
Inclusion criteria comprised patients with indwelling orthopaedic implants—such as plates, screws, intramedullary nails, or prosthetic joints—present in situ for more than two weeks, accompanied by clinical and/or radiological evidence of infection, and willingness to provide consent. Patients with isolated soft tissue infections without implant involvement, those who had received systemic antibiotics within two weeks prior to sampling, and those declining consent were excluded.

Study Size

The sample size of 120 patients was determined based on the average annual caseload of implant-associated orthopaedic infections presenting to the institution. During the preceding year, approximately 55–60 such cases were managed at MGM Hospital. Considering feasibility, available resources, and an anticipated recruitment rate over two years, a sample of 120 consecutive eligible patients was chosen to provide sufficient statistical power for subgroup analyses of microbiological and clinical predictors.

Sample Collection

During revision surgeries or debridement procedures, at least five periprosthetic tissue specimens were collected using separate sterile instruments to minimize cross-contamination. Explanted implants were aseptically placed in sterile containers containing 400 mL of Ringer's solution and transported to the microbiology laboratory within one hour. Implants underwent sonication in an ultrasonic bath at 40 kHz for five minutes to dislodge adherent biofilm-associated bacteria, followed by vortexing for 30 seconds to homogenize the sonication fluid.

Microbiological Processing

Tissue samples were homogenized and inoculated onto blood agar, MacConkey agar, and brain heart infusion broth, incubated aerobically at 37 °C for up to seven days. Sonication fluid aliquots (0.1 mL) were cultured in parallel, with quantitative counts ≥50 colony-forming units per milliliter interpreted as significant growth.
Colonies presumptively identified as staphylococci underwent Gram staining, catalase testing, and tube coagulase testing. Species-level identification was performed using the VITEK 2 automated system (bioMérieux), with confirmation by multiplex PCR targeting species-specific genes and the mecA gene encoding methicillin resistance.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotics tested included oxacillin, vancomycin, linezolid, clindamycin, erythromycin, ciprofloxacin, gentamicin, and rifampicin. Minimum inhibitory concentrations (MICs) for vancomycin and

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linezolid were measured using E-test strips where appropriate.

Biofilm Formation Assay

Biofilm-forming capacity was assessed using a microtiter plate assay. Overnight cultures were standardized to 0.5 McFarland turbidity and inoculated into 96-well polystyrene microplates containing tryptic soy broth supplemented with 1% glucose. After incubation for 24 hours at 37 °C, wells were washed with phosphate-buffered saline, air-dried, fixed with methanol, and stained with 0.1% crystal violet. Excess stain was removed, and dye retained by adherent cells was solubilized in 33% glacial acetic acid. Optical density (OD) was measured at 570 nm. Based on OD values, isolates were classified as non-biofilm producers, moderate biofilm producers, or strong biofilm producers.

Exopolysaccharide Production

Exopolysaccharide synthesis was evaluated using Congo red agar containing sucrose. Black colonies with a dry, crystalline morphology were interpreted as positive for slime production.

Proteolytic Enzyme Activity

Protease production was measured by azocasein hydrolysis. Bacterial supernatants were incubated with 0.5% azocasein at 37 °C for one hour. The reaction was terminated with 10% trichloroacetic acid, and after centrifugation, absorbance at 440 nm was recorded to quantify proteolytic activity.

Data Collection and Clinical Outcomes

Demographic data, comorbidities, implant characteristics, anatomical site, clinical features, and laboratory findings were recorded prospectively. Outcomes evaluated included implant retention or removal, duration of antibiotic therapy, time to infection resolution, and incidence of delayed union or nonunion. All patients were followed for at least six months after treatment completion.

Statistical Analysis

Data were compiled in Microsoft Excel and analyzed using IBM SPSS Statistics version 25. Normality of continuous variables was assessed by the Shapiro–Wilk test. Continuous variables were expressed as means with standard deviations or medians with interquartile ranges, as appropriate, and compared using Student's t-test or the Mann–Whitney U test. Categorical variables were presented as frequencies and percentages and compared using the chi-square test or Fisher's exact test. Associations between continuous variables were evaluated using Spearman's rank correlation coefficient. Multivariate logistic regression analysis was performed to identify independent predictors of implant removal and delayed union. A two-tailed p-value <0.05 was considered statistically significant.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee of Kakatiya Medical College (Approval No.: KIEC/ORTHO/2023/067). Written informed consent was obtained from all participants prior to enrolment. Confidentiality of patient information was strictly maintained, and all procedures were conducted in accordance with the Declaration of Helsinki and local ethical guidelines.

Results

Participant Flow

During the study period, a total of 400 patients with suspected implant-associated orthopaedic infections were screened for eligibility. Of these, 320 were assessed in detail, and 180 patients were excluded due to not meeting the inclusion criteria (isolated soft tissue infections without implants, recent systemic antibiotic use, or refusal to provide consent). A total of 140 patients were confirmed eligible, of whom 20 were excluded for incomplete data or withdrawal of consent prior to surgery. Finally, 120 patients were included in the study and underwent detailed microbiological and biochemical evaluation. All 120 patients completed the six-month follow-up and were included in the final analysis.

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Figure 1. Participant Flow Diagram

Patient Demographics and Clinical Characteristics.

A total of 120 patients with suspected implant-associated infections were enrolled. The mean age was 48.6 ± 15.4 years (range: 19–78), with a male predominance (78 men, 65%). The most frequently involved anatomical sites were long bones of the lower extremities (48.3%), followed by hip and knee arthroplasties (26.6%), upper limb implants (16.6%), and other sites (8.3%). The median duration between implantation and suspicion of infection was seven months (IQR: 4–15 months), reflecting the typically indolent course of these infections (Table 1).

Table 1: Patient Demographics and Clinical Characteristics (N=120)

Characteristic	Value
Age (years), mean ± SD	48.6 ± 15.4
Age range (years)	19 – 78
Sex, n (%)	
• Male	78 (65.0)
• Female	42 (35.0)
Anatomical site of implant, n (%)	
• Lower extremity long bones	58 (48.3)
• Hip and knee arthroplasties	32 (26.6)
• Upper extremity implants	20 (16.6)
• Other sites (ankle, foot)	10 (8.3)
Median time from implantation to infection suspicion (months)	7 (IQR: 4–15)

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Anatomical Distribution of bone Implant-Associated Infections

Figure 2 depicts the distribution of anatomical sites involved in implant-associated infections among the study cohort. Nearly half of the infections (48.3%) occurred in long bones of the lower extremities, underscoring the high risk of infection in weight-bearing bones requiring internal fixation. Hip and knee arthroplasties accounted for 26.6% of cases, reflecting the vulnerability of large joint replacements to chronic infection. Upper extremity implants contributed to 16.6% of infections, while the remaining 8.3% involved other locations such as ankle and foot implants. This distribution highlights the predominance of lower limb procedures in implant-related infections, likely due to both the frequency of surgeries in these regions and the increased mechanical and biological demands on the implants.

Orthopaedic Implant Characteristics and Clinical Parameters

The most common implant type was plates and screws (41.7%), followed by arthroplasty prostheses (26.7%), intramedullary nails (23.3%), and external fixators (8.3%). The majority of infections presented in the delayed period between 3- and 12-months post-implantation (48.3%), whereas early (<3 months) and late (>12 months) infections accounted for 20.8% and 30.8% of cases, respectively. The primary indication for implantation was fracture fixation (65%), with elective arthroplasty procedures comprising 25% and revision surgeries 10%. Regarding comorbidities, diabetes mellitus was present in 18.3% of patients, 28.3% reported a history of smoking, and 5% were receiving immunosuppressive therapy at the time of infection diagnosis (Table 2).

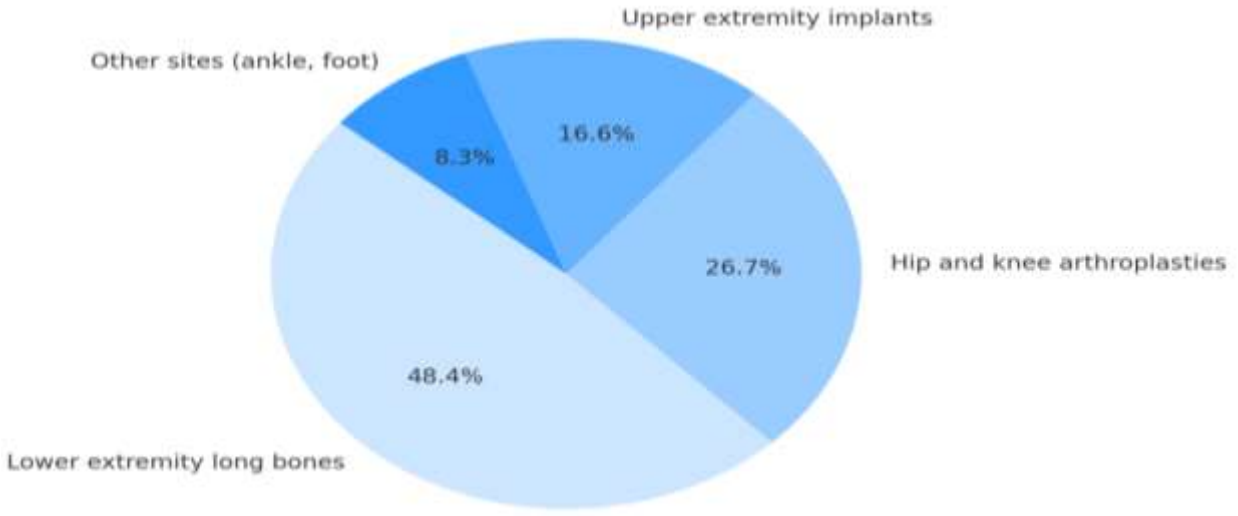

Figure 2: Anatomical Distribution of Implant-Associated Infections

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Table 2: Orthopaedic Implant Characteristics and Clinical Parameters

Characteristic	N (%)
Type of Implant	
Plates and screws	50 (41.7)
Intramedullary nails	28 (23.3)
Arthroplasty prostheses	32 (26.7)
External fixators	10 (8.3)
Timing of Infection	
Early (<3 months)	25 (20.8)
Delayed (3–12 months)	58 (48.3)
Late (>12 months)	37 (30.8)
Indication for Implant	
Fracture fixation	78 (65.0)
Elective arthroplasty	30 (25.0)
Revision surgery	12 (10.0)
Comorbidities	
Diabetes mellitus	22 (18.3)
Smoking history	34 (28.3)
Immunosuppressive therapy	6 (5.0)

Microbiological Culture Results

Culture positivity was high, with 112 of 120 patients (93.3%) yielding growth from either periprosthetic tissue samples or sonication fluid cultures. Among the positive cultures, coagulase-negative staphylococci (CoNS) were isolated in 48 patients, representing 40% of all confirmed infections. Within the CoNS group, *Staphylococcus epidermidis* was by far the predominant species, recovered in 34 cases (70.8%). *Staphylococcus haemolyticus* accounted for 8 cases (16.6%), *Staphylococcus lugdunensis* for 4 cases (8.3%), and other less frequent species, including *Staphylococcus capitis* and *Staphylococcus hominis*, collectively accounted for 2 cases (4.1%). The high isolation rate of *S. epidermidis* underscored its significance as an opportunistic pathogen in implant-associated infections.

Methicillin resistance was common among CoNS isolates, with 33 of the 48 strains (68.7%) demonstrating oxacillin resistance confirmed by cefoxitin screening and mecA gene detection in representative isolates (Table 3). Notably, methicillin resistance was slightly more prevalent in *S. epidermidis* (73.5%) compared to non-*epidermidis* species (56.2%), though this difference was not statistically significant.

Table 3: Distribution of CoNS Species Isolated (n=48)

Species	Number (%)
Staphylococcus epidermidis	34 (70.8)
Staphylococcus haemolyticus	8 (16.7)
Staphylococcus lugdunensis	4 (8.3)
Other species	2 (4.1)

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Antimicrobial Susceptibility

The antimicrobial susceptibility profiles of the CoNS isolates revealed widespread multidrug resistance. Resistance rates were highest for erythromycin (79.1%) and ciprofloxacin (64.5%). Clindamycin resistance was observed in 60.4% of isolates, while gentamicin resistance was noted in 35.4%. Rifampicin resistance remained relatively low (18.7%), and all isolates were uniformly susceptible to vancomycin and linezolid. Among methicillin-resistant isolates, multidrug resistance, defined as resistance to at least three antibiotic classes, was detected in 28 of 33 strains (84.8%). This finding emphasizes the therapeutic challenges in managing these infections. The minimum inhibitory concentrations (MICs) for vancomycin ranged from 0.5–2 µg/mL, while linezolid MICs were ≤2 µg/mL in all tested isolates, supporting their role as effective treatment options (Table 4).

Table 4: Antibiotic Resistance Patterns among CoNS Isolates

Antibiotic	Resistant Isolates (%)
Oxacillin	68.7
Erythromycin	79.1
Clindamycin	60.4
Ciprofloxacin	64.5
Gentamicin	35.4
Rifampicin	18.7
Vancomycin	0
Linezolid	0

Biofilm Formation and Biochemical Characteristics

Assessment of biofilm-forming capacity using the microtiter plate assay demonstrated that strong biofilm production (optical density >0.240) was present in 38 isolates (79.1%). Moderate biofilm formation was observed in 7 isolates (14.5%), while only 3 isolates (6.2%) were categorized as non-biofilm producers. Exopolysaccharide production, assessed by Congo red agar morphology, was positive in 42 isolates (87.5%), consistent with their robust biofilm matrix synthesis. In terms of biochemical activity, proteolytic enzyme production measured by azocasein hydrolysis ranged widely among isolates, with absorbance values spanning from 0.25 to 1.85 (mean ± SD: 1.06 ± 0.42), indicating considerable variability in extracellular protease expression.

Figure 3 depicts the distribution of biofilm formation categories, illustrating the predominance of strong biofilm producers among clinical isolates. These results highlight the critical role of biofilm and extracellular matrix components in the persistence of infection on implant surfaces.

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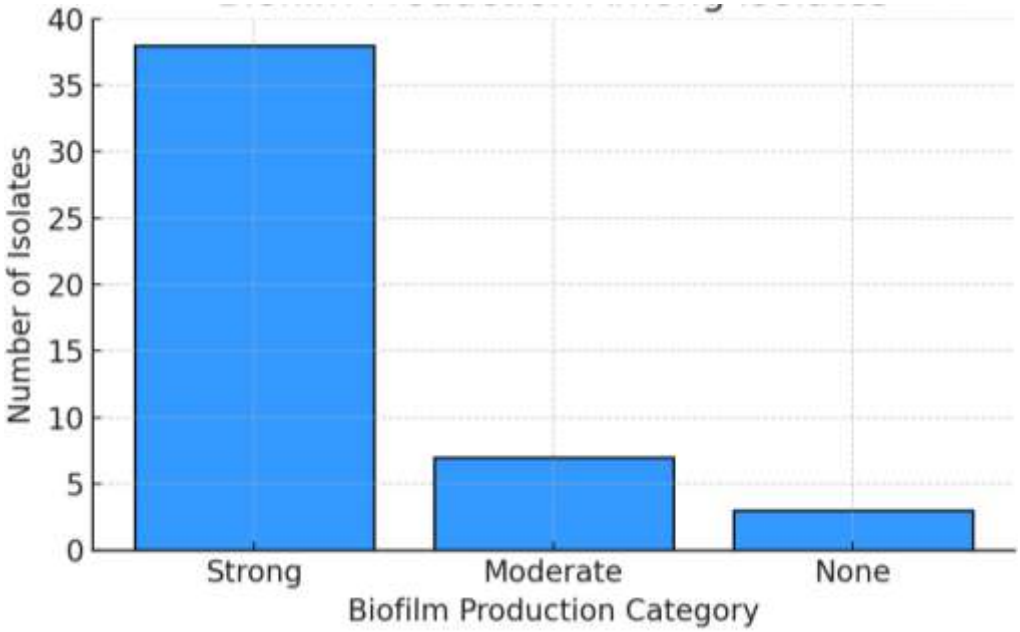
Figure 3: Distribution of biofilm-forming categories



Association with Clinical Outcomes

Correlation of microbiological and biochemical characteristics with clinical outcomes revealed important trends. Among patients infected with strong biofilm-producing strains (n=38), implant removal was necessary in 28 cases (73.6%), compared to only 3 of 10 cases (30%) in those infected with moderate or non-biofilm-producing strains. This association between biofilm formation and the need for implant removal was statistically significant (p=0.004). The mean duration of intravenous and oral antibiotic therapy was significantly prolonged in patients infected with strong biofilm-forming CoNS, averaging 6.4 ± 2.1 weeks, whereas patients with weaker biofilm producers required a mean of 4.2 ± 1.6 weeks of therapy (p=0.001). Delayed union or nonunion occurred in 17 of 38 cases (44.7%) in the strong biofilm group, compared with 2 of 10 cases (20%) in the other group (p=0.03), demonstrating the deleterious impact of biofilm on bone healing (Table 5; Figure 4).

Table 5: Association of Biofilm Production with Clinical Outcomes

Outcome	Strong Biofilm (n=38)	Others (n=10)	p-value
Implant removal required	28 (73.6%)	3 (30%)	0.004
Delayed union/nonunion	17 (44.7%)	2 (20%)	0.03
Mean antibiotic duration (weeks)	6.4 ± 2.1	4.2 ± 1.6	0.001

Additionally, subgroup analysis revealed that methicillin-resistant CoNS were associated with longer hospital stays and increased rates of multidrug-resistant profiles, though no statistically significant difference in implant removal rates was observed between methicillin-resistant and methicillin-susceptible strains.

No mortality was recorded during the follow-up period. All patients were followed for at least six months after

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completion of treatment, during which no recurrence of infection was observed in patients who underwent implant removal and prolonged antibiotic therapy.

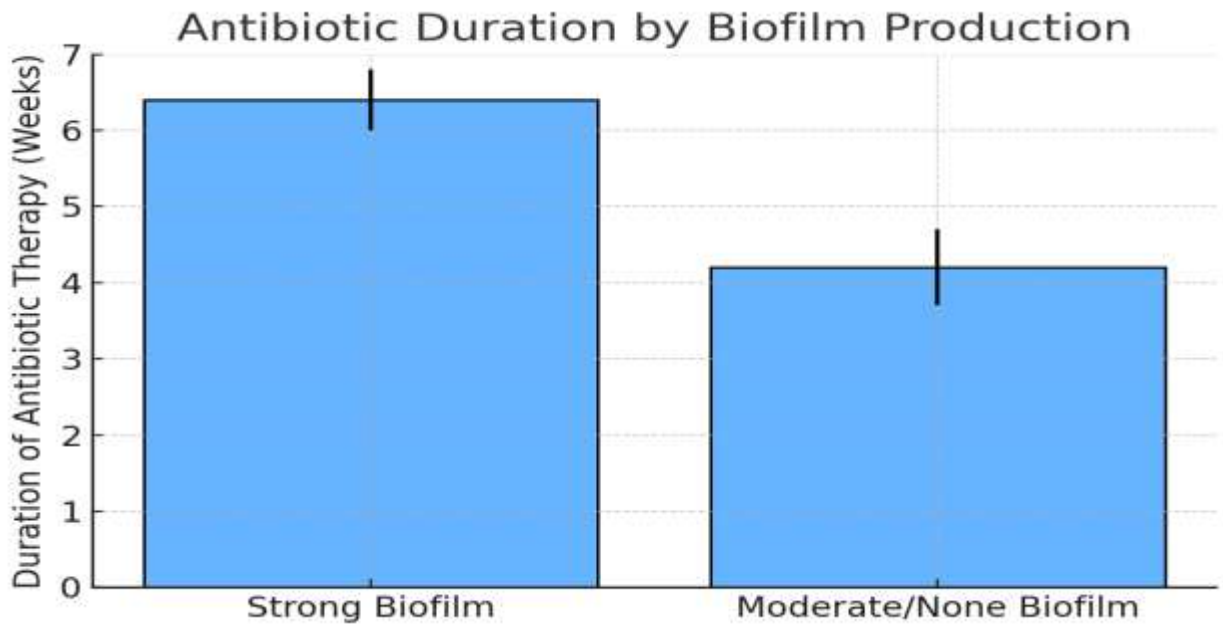

Figure 4: Mean Antibiotic Duration by Biofilm Category (± SE)

Multivariate Analysis of Predictors of Implant Removal

The multivariate logistic regression model was performed to identify factors independently associated with the need for implant removal in patients with coagulase-negative staphylococcal infections. Strong biofilm production emerged as a statistically significant predictor, with an odds ratio of 4.25 (95% CI: 1.32–13.69; p=0.015), indicating that infection with strong biofilm-forming strains increased the likelihood of implant removal more than fourfold compared to moderate or non-biofilm producers, after controlling for other variables.

Proteolytic enzyme activity also showed a significant association, with each unit increase in enzyme production nearly doubling the odds of implant failure (OR 1.92; 95% CI: 1.03–3.57; p=0.040). This finding underscores the role of extracellular enzymes in promoting persistent infection and tissue damage.

In contrast, methicillin resistance (OR 1.84; 95% CI: 0.61–5.58; p=0.275) and infection with *S. epidermidis* species (OR 1.46; 95% CI: 0.43–4.93; p=0.543) were not statistically significant predictors in the adjusted model, suggesting that the presence of multidrug resistance or a specific species alone did not independently increase the risk of implant removal (Table 6).

Overall, these results highlight that biofilm-associated virulence characteristics, particularly robust biofilm formation and proteolytic enzyme production, were the primary drivers of treatment failure and surgical intervention, rather than antibiotic resistance alone. This emphasizes the need for targeted strategies to disrupt

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biofilm integrity and manage chronic implant infections effectively.

Table 6: Logistic Regression Predicting Implant Removal

Variable	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
Strong biofilm production	4.25	1.32–13.69	0.015
Methicillin resistance	1.84	0.61–5.58	0.275
S. epidermidis species	1.46	0.43–4.93	0.543
Proteolytic enzyme activity (per unit)	1.92	1.03–3.57	0.040

Multivariate Analysis of Predictors of Delayed Union or Nonunion

Table 7 presents the results of logistic regression evaluating independent predictors of delayed union or nonunion among patients with implant-associated coagulase-negative staphylococcal infections. Strong biofilm production was the only variable that reached statistical significance, with an odds ratio of 3.12 (95% CI: 1.01–9.61; p=0.048), indicating that patients infected with strong biofilm-forming strains had over three times the odds of experiencing impaired bone healing compared to those with moderate or non-biofilm-producing strains. Although higher proteolytic enzyme activity showed a trend toward increased risk (OR 1.54; 95% CI: 0.87–2.71), this association did not reach statistical significance (p=0.133). Methicillin resistance (OR 1.29; 95% CI: 0.44–3.77; p=0.647) and infection with *S. epidermidis* species (OR 1.12; 95% CI: 0.34–3.65; p=0.854) were not significantly associated with delayed union or nonunion in the adjusted model. These findings suggest that strong biofilm production is the main independent contributor to delayed bone healing, reinforcing the clinical importance of biofilm-related virulence in orthopaedic implant infections

Table 7: Logistic Regression Predicting Delayed Union/Nonunion

Variable	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
Strong biofilm production	3.12	1.01–9.61	0.048
Methicillin resistance	1.29	0.44–3.77	0.647
S. epidermidis species	1.12	0.34–3.65	0.854
Proteolytic enzyme activity (per unit)	1.54	0.87–2.71	0.133

Correlation Between Biofilm Density and Antibiotic Duration

Table 8 summarizes the correlation analysis between continuous biofilm optical density measurements and the duration of antibiotic therapy. Spearman's rank correlation coefficient demonstrated a moderate positive association (rho = +0.56, p <0.001), indicating that higher biofilm density was significantly correlated with longer courses of antibiotic treatment. This suggests that as the degree of biofilm formation increased, patients required more prolonged antimicrobial therapy to achieve infection control, further emphasizing the clinical impact of robust biofilm production in implant-associated infections.

Table 8: Correlation Between Biofilm Optical Density and Antibiotic Duration

Variable Pair	Spearman's rho	p-value
Biofilm OD (continuous) vs. antibiotic duration	+0.56	<0.001

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Discussion

This study demonstrates that coagulase-negative staphylococci (CoNS) are clinically significant pathogens in orthopaedic implant-related infections, with biofilm formation and proteolytic activity emerging as key predictors of adverse outcomes. The interpretation of these findings suggests that the persistence of infection and implant failure is not solely dependent on antimicrobial resistance, but rather on the virulence mechanisms that enable CoNS to colonize biomaterials, evade host immunity, and impair bone healing. The high rates of implant removal, prolonged antibiotic therapy, and delayed union associated with strong biofilm producers underscore the central role of biofilm in driving chronicity and poor clinical outcomes. Similarly, the independent association between elevated proteolytic enzyme activity and implant failure indicates that enzymatic tissue damage contributes substantially to infection persistence. Together, these results indicate that therapeutic strategies should extend beyond conventional antimicrobial resistance considerations and target biofilm-associated virulence pathways to improve treatment outcomes.

When compared with published data, the incidence of CoNS infections in this study (40% of all implant-associated infections) aligns with reports identifying CoNS among the most frequent pathogens in prosthetic joint and fracture-fixation device infections [1,2]. Staphylococcus epidermidis was the predominant species, representing more than 70% of isolates, consistent with its recognized ability to adhere to abiotic surfaces and establish chronic biofilm-mediated infections [3,11–13]. The methicillin resistance rate of nearly 69% also parallels large surveillance studies, where resistance rates in S. epidermidis have exceeded 70% [3,8,14–17]. Multidrug resistance against commonly used antibiotics, including erythromycin, ciprofloxacin, and clindamycin, was frequent, while all isolates remained susceptible to vancomycin and linezolid. However, reliance on these last-resort agents may be problematic due to reduced activity against biofilm-embedded cells and concerns about emerging intermediate susceptibility [4,8].

Importantly, this study highlights that biofilm-related virulence factors were stronger determinants of treatment outcomes than methicillin resistance. Strong biofilm formation correlated with longer antibiotic duration, increased implant removal, and impaired bone healing. Multivariate analysis further identified strong biofilm production as an independent predictor of implant removal, with a more than fourfold higher likelihood of explantation. These findings strengthen previous evidence linking biofilm to chronic inflammation, poor osseointegration, and delayed fracture healing [3,9,10]. Proteolytic enzyme production, although less commonly evaluated, also emerged as a relevant virulence determinant. Higher enzyme activity was independently associated with implant removal, supporting experimental data that staphylococcal proteases contribute to tissue destruction, immune evasion, and biofilm maturation [18].

From a clinical perspective, most infections occurred in association with plates, screws, and intramedullary nails, which are frequently used in fracture fixation. Nearly half were delayed infections (3–12 months after surgery), consistent with the chronic, indolent nature typical of biofilm-mediated infections. These findings emphasize the need for heightened clinical suspicion in patients presenting with subtle symptoms several months postoperatively. Diagnostic challenges remain due to the ubiquity of CoNS as skin commensals. In this study, the use of implant sonication significantly improved culture yield, achieving positivity in over 93% of cases, which is in line with reports showing superior sensitivity compared to conventional tissue cultures [9,19].

Generalizability

The findings of this study provide valuable insights into the role of coagulase-negative staphylococci in orthopaedic implant infections and are likely generalizable to tertiary care centres in resource-limited regions where implant-related infections and multidrug resistance are prevalent. However, caution should be applied when extrapolating results to populations with different healthcare systems, surgical practices, or antimicrobial stewardship policies. Broader multicentre studies with molecular epidemiological approaches and longer-term follow-up are warranted to validate and extend these observations.

Conclusion

This study underscores the significant role of coagulase-negative staphylococci as important opportunistic

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pathogens contributing to orthopaedic implant failure. Our findings demonstrate that Staphylococcus epidermidis remains the predominant species involved in these infections, characterized by a high prevalence of methicillin resistance and widespread multidrug resistance patterns that complicate empirical antibiotic selection. Beyond resistance, the robust biofilm-forming capacity and exopolysaccharide production observed in most isolates highlight the pivotal role of biofilm in driving chronicity, immune evasion, and treatment failure. Importantly, strong biofilm production and elevated proteolytic enzyme activity emerged as independent predictors of adverse outcomes, including increased rates of implant removal, prolonged antibiotic therapy, and delayed bone healing. These observations reinforce the necessity for early recognition, comprehensive microbiological diagnosis incorporating techniques such as implant sonication, and an integrated management strategy that combines prompt surgical intervention with targeted antimicrobial therapy. Future research should prioritize the molecular characterization of biofilm-associated virulence mechanisms, the development of innovative anti-biofilm and anti-virulence therapies, and prospective studies evaluating long-term clinical and functional outcomes to optimize the management of patients with implant-associated CoNS infections.

Limitations

This study has several limitations that should be considered when interpreting the findings. First, it was conducted at a single tertiary care center in India, which may limit the applicability of results to other geographic regions with different microbial epidemiology and antibiotic usage patterns. Second, molecular typing methods, such as multilocus sequence typing or whole-genome sequencing, were not performed, preventing detailed assessment of clonal relationships and transmission dynamics among isolates. Third, the study did not evaluate host-related factors such as immunosuppressive status, nutritional parameters, or comorbidities in detail, which could influence infection outcomes. Fourth, although follow-up extended for six months after treatment completion, longer-term recurrence rates and functional outcomes were not assessed. Finally, biofilm formation was assessed in vitro, which may not fully replicate in vivo conditions on implant surfaces.

Recommendations

Based on the findings of this study, several important recommendations can be proposed to enhance the management of coagulase-negative staphylococcal implant-associated infections. Routine incorporation of implant sonication into diagnostic protocols is strongly advised to improve the detection of biofilm-embedded pathogens and ensure accurate microbiological identification. Laboratories should consider implementing quantitative biofilm assessment techniques, as the presence of robust biofilm production was shown to be a significant predictor of adverse outcomes, including implant removal and delayed bone healing. Given the high prevalence of methicillin resistance and multidrug-resistant phenotypes observed among isolates, empirical antimicrobial therapy should be approached cautiously, with prompt adjustment based on culture and susceptibility results. Infections caused by strong biofilm-producing strains may warrant early and decisive surgical intervention, including implant removal or revision, to achieve effective infection clearance and optimize recovery. Additionally, stringent adherence to perioperative aseptic measures and evidence-based prophylactic strategies should be reinforced to prevent implant colonization. Future research should prioritize molecular investigations into biofilm-related virulence determinants and proteolytic enzyme expression to facilitate the development of innovative anti-biofilm therapies. Finally, extending patient follow-up beyond six months is recommended to monitor for late recurrences and to more comprehensively evaluate long-term clinical and functional outcomes.

Abbreviations

CoNS: Coagulase-negative staphylococci
PDX: Patient-derived xenograft
MRCoNS: Methicillin-resistant coagulase-negative staphylococci
MSSA: Methicillin-susceptible Staphylococcus aureus
MIC: Minimum inhibitory concentration
BSL: Biosafety level
ELISA: Enzyme-linked immunosorbent assay
OD: Optical density
PCR: Polymerase chain reaction
CLSI: Clinical and Laboratory Standards Institute
cGMP: Current good manufacturing practice

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GLP: Good laboratory practice
SD: Standard deviation
IQR: Interquartile range
OR: Odds ratio
CI: Confidence interval

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest relevant to this study.

Authors Contributions

KRB conceived the study design, supervised data collection, and contributed to manuscript drafting. URV performed microbiological experiments, data analysis, and interpretation of results. RK provided critical revisions, contributed to the statistical analysis, and guided the discussion of findings. LC assisted with laboratory assays, literature review, and preparation of figures and tables. All authors reviewed and approved the final version of the manuscript.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. All relevant data supporting the conclusions of this article can be provided upon request for purposes of academic research and verification.

Biography

Dr. Keshava Rao Bolloju, MS is a distinguished orthopaedic surgeon currently serving as an Associate Professor in the Department of Orthopaedics at Government Medical College, Narsampet, Telangana. He also functions as a National External Assessor for the National Quality Assurance Standards (NQAS) Programme, contributing to the quality assessment and accreditation of government hospitals across India. Previously, he held the position of Assistant Professor at MGM Hospital and Kakatiya Medical College, Warangal, where he contributed extensively to both clinical services and academic mentorship. Dr. Bolloju earned his Bachelor of Medicine and Bachelor of Surgery (M.B.B.S.) from Kakatiya Medical College, Hanumakonda, Warangal (2000–2005), followed by a Master of Surgery (M.S.) in Orthopaedics from the Prathima Institute of Medical Sciences, Karimnagar, Telangana (2012–2015). With over a decade of dedicated service spanning primary, secondary, and tertiary healthcare settings, he has demonstrated exceptional proficiency as a treating physician, medical officer, and programme officer for national health initiatives throughout the erstwhile state of Andhra Pradesh and present-day Telangana. Renowned for his surgical acumen, Dr. Bolloju possesses advanced expertise in managing complex trauma, performing joint reconstructions, and employing Ilizarov's technique of external fixation for limb salvage and correction of severe deformities. His surgical repertoire encompasses the management of polytrauma, open fractures, neglected injuries, and deformity corrections, delivered with a patient-centred approach that emphasises functional recovery and quality of life. He has been instrumental in training and mentoring undergraduate and postgraduate medical students, integrating evidence-based practices into orthopaedic education. His primary research interests include trauma care protocols, general orthopaedics, and innovations in external fixation techniques. Dr. Bolloju has organised numerous outreach health camps, delivering specialised care to individuals with disabilities and facilitating the issuance of disability certificates to support their social and economic empowerment. He has authored and co-authored multiple research publications in reputable indexed journals, including EMBASE, SCOPUS, and

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