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# Comparative analysis of *in vitro* biofilm formation in gram-positive and gram-negative pathogens from orthopaedic implant infections: a retrospective cross-sectional study in a tertiary care centre

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## ABSTRACT

**Background:** Orthopaedic implant infections (ODRI) remain a significant clinical challenge due to their association with persistent biofilm formation, which complicates treatment. 1.2 Objective: This study aimed to investigate the biofilm-forming potential of Gram-positive and Gram-negative pathogens isolated from orthopaedic implant infections in a tertiary care setting.
**Methods:** A retrospective cross-sectional study was conducted at the Krishna Institute of Medical Sciences, Secunderabad, India, from February 2023 to January 2024. Clinical samples from orthopaedic implant infections, were processed for bacterial culture and biofilm formation using the Tissue Culture Plate (TCP) method in triplicates. Bacterial identification was performed using the Vitek 2 Compact system.
**Results:** Of 87 patients diagnosed with orthopaedic implant infections, 62 (71.26%) were culture-positive, with 35 (56.45%) Gram-negative bacilli and 27 (43.54%) Gram-positive cocci. Biofilm formation was observed in 59.25% of Gram-positive isolates, with 18.51% strong biofilm producers, 40.74% moderate producers, and 40.74% weak/non-producers. Among Gram-negative isolates, 31.42% were biofilm producers, with 5.71% strong, 25.71% moderate, and 68.57% weak/non-producers. A higher prevalence of biofilm production was noted in Gram-positive organisms compared to Gram-negative bacteria.
**Conclusion:** The study highlights the higher propensity of Gram-positive bacteria to form biofilms, which may contribute to the persistence and chronicity of orthopaedic implant infections.

**Keywords:** Orthopaedic implant infections, Biofilm production, Gram positive

## INTRODUCTION

Biofilms are organized communities of multiple species of bacteria/ fungi embedded in an organic polymer matrix of extracellular DNA (fragments of damaged cell genome), proteins and polysaccharides attached to the surface, known as extracellular polymeric substances (EPS), often labelled as the "house of the biofilm cells".[1-2] Formation of biofilm is a complex process. The several stages being adherence, accumulation, maturation and detachment.[2] Biofilm producing bacteria are responsible for many recalcitrant infections. They exhibit resistance to antibiotics by various methods like restricted permeability due to glycocalyx, decreased growth rate and expression of resistance genes.[4] Biofilm bacteria can survive up to 1500 (typically 100 to 250) times the amount of an antibody needed to kill the same bacteria growing in a liquid culture.[5] Organisms producing biofilms *Staphylococcus aureus*, Coagulase Negative Staphylococci (CoNS), *Enterococcus faecium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, Citrobacter, Proteus, etc.

The objective of this study was to investigate the biofilm-forming potential of Gram-positive and Gram-negative pathogens isolated from orthopaedic implant infections in a tertiary care setting.

## METHODS

The retrospective, cross-sectional study was conducted in the Department of Microbiology and Department of Laboratory Sciences at Krishna Institute of Medical Science, Secunderabad, Telangana, India, from February 2023 to January 2024, following approval from the Scientific and Ethics Committee on January 9th, 2023.

Clinical samples of orthopaedic implant infections including pus, intraoperative tissues and removed implants were collected and inoculated in BSL-2 cabinet. Plates were kept for extended incubation of 5 days. Bacterial identification, if any, was performed using the GP and GN cards of the Vitek 2 Compact system (bioMérieux). Biofilm formation in positive cultures was detected using Tissue Culture Plate (TCP) method in triplicates as described by Christensen et al, as depicted in Figure 1.[6]

### *Statistical analysis*

Statistical analysis was carried out using IBM SPSS.

### *Ethical consideration*

Approval was obtained from Institutional Ethical Committee before the commencement of the study.

## RESULTS

During the study period, clinical diagnosis of ODRI was made in 87 patients. Of these, 62 were culture positive. Thus, the culture positivity rate was calculated to be 71.26%. Among the 62 culture positive samples, 35 were Gram negative bacilli (56.45%) and 27 were Gram positive cocci (43.54%). Spectrum of organisms isolated is illustrated in Table 1.

**Table 1: Spectrum of organisms isolated (n=62).**

| Gram positive cocci (n=27, 43.48%) | | | Gram negative bacilli (n=35, 56.45%) | |
|---|---|---|---|---|
| **Organism** | | **Number** | **Organism** | **Number** |
| ***Staphylococcus aureus*** | | 19 | *Klebsiella pneumoniae* | 11 |
| **CoNS** | *Staphylococcus epidermidis* | 5 | *Pseudomonas aeruginosa* | 7 |
| | *Staphylococcus haemolyticus* | 1 | *Escherichia coli* | 6 |
| ***Streptococcus agalactiae*** | | 1 | *Enterobacter cloacae* | 4 |
| ***Streptococcus gallolyticus*** | | 1 | *Proteus mirabilis* | 4 |
| | | | *Pseudomonas stutzeri* | 1 |
| | | | *Stenotrophomonas maltophilia* | 1 |
| | | | *Morganella morganii* | 1 |

Among the 27 Gram positive isolates, 18.51% were strong biofilm producers, 40.74% were moderate biofilm producers, and 40.74% were weak/ non-biofilm producers, depicted in Figure 2.

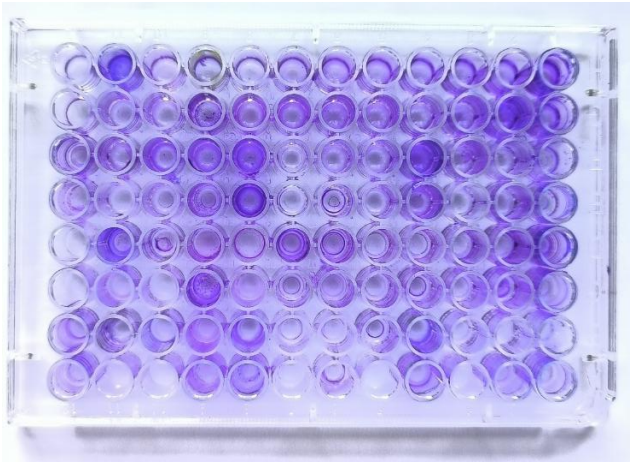


**Figure 1: Detection of in vitro biofilm formation by Tissue culture method.**

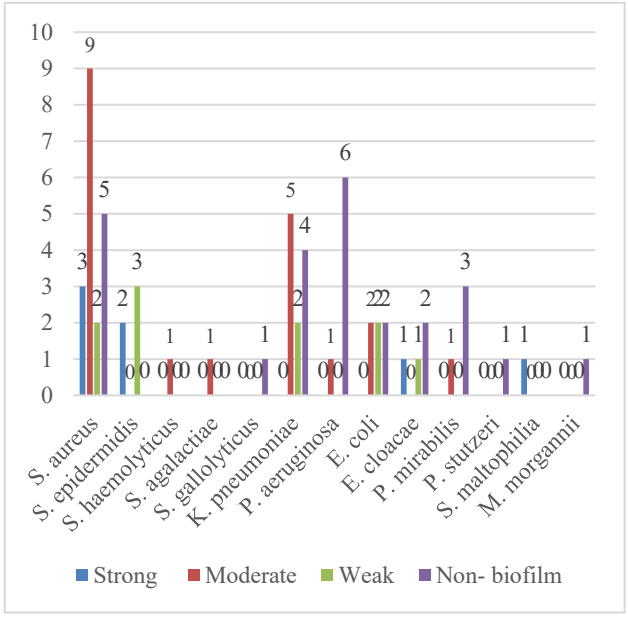



**Figure 2: Prevalence of biofilm in GP and GN bacteria.**

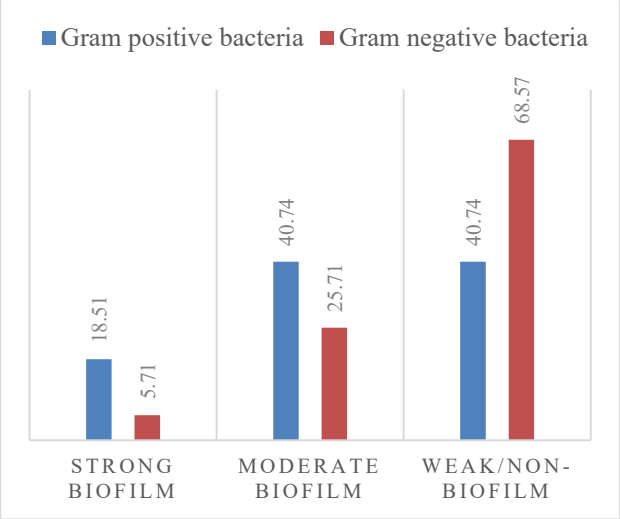
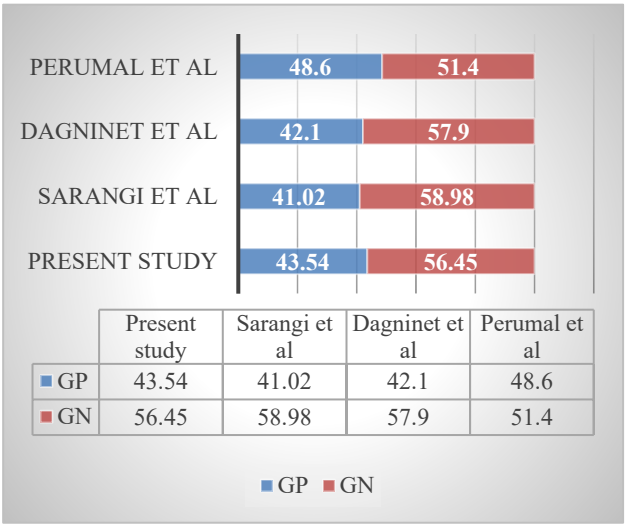



**Figure 3: Comparison between GP and GN biofilm formation.**




| | Present study | Sarangi et al | Dagninet et al | Perumal et al |
|---|---|---|---|---|
| GP | 43.54 | 41.02 | 42.1 | 48.6 |
| GN | 56.45 | 58.98 | 57.9 | 51.4 |

**Figure 4: Spectrum of GP and GN bacteria isolated in various studies.**

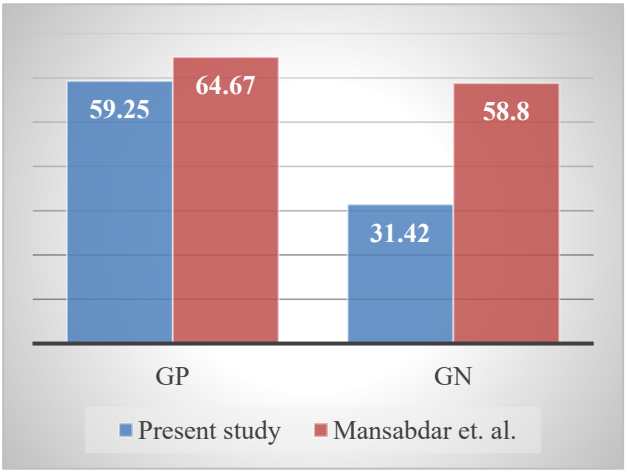



**Figure 5: Comparison of biofilm producers of GP and GN bacteria in various studies.**

Among the 27 Gram negative isolates, 5.71% were strong biofilm producers, 25.71% were moderate biofilm producers, and 68.57% were weak/ non-biofilm producers. This is represented in Figure 2. When comparing the prevalence of biofilm production in Gram-positive and Gram-negative bacteria, it was observed that Gram positive bacteria were more prone to form biofilm, pictorially represented in Figure 3.

## DISCUSSION

In the present study, 56.45% isolates were *Gram negative bacilli*, and was comparable to the findings of Sarangi et al, Alelign et al and Perumal et al, as shown in Figure 4.[7-9]

However, the most common isolate was *Staphylococcus aureus* followed by *Klebsiella pneumoniae* which accounted for 19 and 11 out of the 62 culture positives, respectively, in this study. This correlates with the findings of Alelign et al, Perumal et al, and Fernandez et al.[8-10] They reasoned that *Staphylococcus spp*. are an integral part of the normal transient and resident flora of the skin, which might have transmitted endogenously to the surgical site either during trauma or the operative procedure itself. Also, *Staphylococcus* spp. might spread exogenously from axillary or nasal HCW carriers, or may spread from the environment (bed linen, dressings, surgical instruments).[11-13] This could also be the reason for majority of *Staphylococcus* spp being isolated from ODRI patients in the present study.

As depicted in Figure 5, in the present study, 59.25% of Gram-positive isolates while 31.42 of Gram-negative isolates were biofilm producers. About 73.68% of isolates of *Staphylococcus aureus* formed biofilm followed by 63.63% of isolates of *Klebsiella pneumoniae*. This percentage was lower than that of Medegar et al where 67.64% and 58.8% of Gram-positive and Gram-Negative isolates were biofilm producers.[12] This lower percentage in the present study could be due to the difference in strains prevalent in the different geographical locations, nature of surgeries, and variations in the infection control practices of the different hospitals.

Despite this difference, in both the studies biofilm formation was noted more in Gram-positive organisms than in the Gram-negative organisms. This conclusion is supported by Ruhal et al, who observed that biofilm formation in Gram-positive bacteria can be influenced by environmental conditions and may involve polysaccharide-dependent or -independent regulation. Conversely, Gram-negative bacteria typically require higher intracellular concentrations for initial adhesion compared to Gram-positive bacteria.[13]

This study had several limitations. Being a single-centre study with a relatively small sample size, the findings may not be generalizable. Biofilm formation was assessed only in vitro using the Tissue Culture Plate method, which may not fully represent in vivo conditions. Molecular characterization of biofilm-associated genes and

correlation with antibiotic resistance or clinical outcomes were not performed. Additionally, only culture-positive cases were included, possibly excluding infections caused by fastidious organisms.

## CONCLUSION

In the study, it was found that among the Gram-positive isolates, 18.51% were strong biofilm producers, 40.74% were moderate producers, and 40.74% were weak/non-producers. In comparison, among Gram-negative isolates, 5.71% were strong producers, 25.71% were moderate producers, and 68.57% were weak/non-producers. The study concluded that Gram-positive bacteria were more prone to forming biofilms than Gram-negative bacteria.

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*Ethical approval: The study was approved by the Institutional Ethics Committee*

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