Investigate the Prevalence & Mechanisms of Biofilm Formation in Indwelling devices (e.g., catheters, prosthetics) & its Role in Persistent Infection and the Efficacy of Novel Anti-Biofilm Strategies or Coatings.

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ABSTRACT:

Objective: To evaluate the biofilm formation by Staphylococcus aureus along with Pseudomonas aeruginosa on silicone and polyurethane surfaces as well as to evaluate the antimicrobial efficacy of polymer-based coatings in reducing biofilm adherence and associated complications.

Methodology: For research we used in vitro model to investigate biofilm formation by S. aureus and P. aeruginosa on silicone and polyurethane surfaces. The reduction of bacterial adhesion, extracellular polymeric substances (EPS) production and the total biofilm density was evaluated for polymer-based antimicrobial coatings. We performed microscopic analysis along with viability testing to confirm the results.

Results: The results were very different after using these coatings. *P. aeruginosa* made more biofilms than *S. aureus*, and silicone surfaces kept biofilms with high biomass levels. Polymer-based antimicrobial coatings worked very well to stop biofilm from forming and sticking. Microscopic analysis and viability assessment verified a substantial reduction in biofilm density on coated surfaces. The coatings also made it harder for bacteria to stick to surfaces and made less EPS.

Conclusion: The results show how important it is to have better material engineering and polymer-based antimicrobial coatings to reduce infections related to biofilms in medical implants. Future studies should focus on improving the formulation of the coatings and evaluate extended use of antibiotics in clinical settings. Sophisticated preventive mechanisms could help to increase the service life and safety of in-vivo medical implants.

Keywords: bacterial adhesion, biofilm, indwelling devices, catheter infections, and antimicrobial coatings.

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Introduction:

In medical science, new surgical and medical devices introduced such as urinary catheters, prosthetic joints, central venous catheters (CVP) have altered modern-day medicine and empowered long-term patient care. For several diseases like chronic diseases, the following surgery, and prolonged therapies like chemotherapy, radiotherapy, dialysis. These new modernized devices offer critical medical support. However, prolonged usage is directly linked to the infection, it is difficult to treat and result in prolonged chronic ailment and side effects like device failure and sepsis. So biofilms are very resistant and lethal.[1] Biofilms developed when Bacterial colonies attached to the surface and produced large number of EPS (extracellular polymeric substances) by this ability bacteria developed highly resistance matrix that unable bacteria to resist to medication and human immunity. Biofilms are highly resistant and extremely challenging to treat because of EPS produced by them. Sometimes new group of antibiotics are also ineffective against biofilms. Biofilms mostly associated with *Staphylococcus aureus* and *Pseudomonas aeruginosa* . On a variety of surfaces, such as silicone, polyurethane, and polytetrafluoroethylene (PTFE) utilized in medical devices, they may produce robust biofilms.[2,3]

Infections caused by biofilm not only effect patient's health but also it plays a vital role in prolonged hospital stay, increase health cost and morbidity respectively.[4] Old regime of antibiotics and traditional use of antibiotics are not helpful in eradication of biofilms because of highly resistance nature. However advance and novel approach can resolve this issue. we can use antimicrobial coatings, surface modifications, and inventive drug delivery systems.[2] This study aims to quantify biofilm growth on different device materials and analyze the efficacy of antimicrobial coating in biofilm associated infections.

Methodology:

Bacterial Strains and Culture Conditions:

Two strains staphylococcus aureus (ATCC 25923) and Pseudomonas aeruginosa (ATCC 27853) were used in this study. Bacterial strains were cultured in TSB agar before testing for biofilm, culture was done at 37°C in aerobic environment.

Indwelling Device Materials:

We have used three medical-grade materials to study biofilms. These were poly tetra fluoroethylene (PTFE), polyurethane and silicon. Process started with cutting of these

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materials into identical sizes and then inoculated with bacteria. After that uncoated control samples and the samples coated with antibiotics examined. Coated material was silver nanoparticles.

Biofilm Formation Assay:

In order to measure biofilm production ,we have used crystal violet staining technique.24-well plates containing the sterile material discs were supplemented with bacterial suspensions at a concentration of 10^6 CFU/ml. To encourage the formation of biofilm, the plates were incubated for 48 hours at 37°C. To get rid of any non-adherent cells, the wells were rinsed with phosphate-buffered saline (PBS) after incubation.

0.1% Crystal violet solution were used to stain biofilms for 15 min and remaining washed out with sterile water. We have used 95% ethanol to dissolved the dye and by using the absorbance at OD600 the biomass of the biofilm was determined.

Quantitative PCR (qPCR) Analysis:

Total RNA was isolated from bacteria that formed biofilms using the TRIzol reagent in order to measure the expression of genes linked to biofilms. A reverse transcriptase enzyme was used to synthesize complementary DNA (cDNA). Using particular primers that target biofilm-related genes (pelA for *P. aeruginosa* and icaA for *S. aureus*), quantitative PCR (qPCR) was carried out. Using 16S rRNA as the reference gene, relative gene expression was normalized and examined using the ΔΔCt technique.

Statistical Analysis:

One-way analysis of variance (ANOVA) and Tukey's post hoc test for multiple comparisons were used to analyze the data. Statistical significance was defined as a p-value of less than 0.05. Every experiment was carried out in triplicate, and the mean ± standard deviation is used to represent the results.

Rationale of study:

Biofilm formation on indwelling medical devices like catheters and prosthetics is a big reason why healthcare-associated infections don't go away. Microorganisms in biofilms become very resistant to antibiotics and the immune system of the host. This can cause infections to come back, longer hospital stays, and device failure. This research holds great importance in understanding the prevalence, mechanism of formation of biofilm and the evaluation of the anti-biofilm properties of innovative new coatings and anti-biofilm prevention strategies to improve patient outcomes.

Results:

The findings showed that, in contrast to silicone and PTFE, Pseudomonas aeruginosa produced noticeably denser biofilms on polyurethane surfaces. The biofilm biomass of Staphylococcus aureus, on the other hand, was consistently smaller than that of P. aeruginosa, despite the fact that it showed substantial biofilm formation across all tested substrates. According to these results (Table 1), polyurethane offers a surface that is more conducive to bacterial adhesion and biofilm formation, potentially raising the risk of infection in indwelling medical devices. Formation of biofilms on various device materials is shown in table no 1.

Table 1. Staphylococcus aureus and Pseudomonas aeruginosa form biofilms on medical-grade materials.

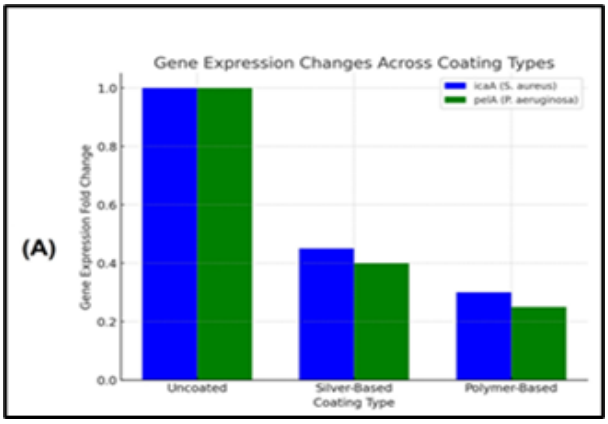
Bacterial Strain	Polyurethane (OD600)	Silicone (OD600)	PTFE (OD600)
Staphylococcus aureus	0.60 ±0.04	0.45 ±0.05	0.52 ±0.03
Pseudomonas aeruginosa	1.10 ±0.08	0.70 ±0.06	0.85 ±0.05

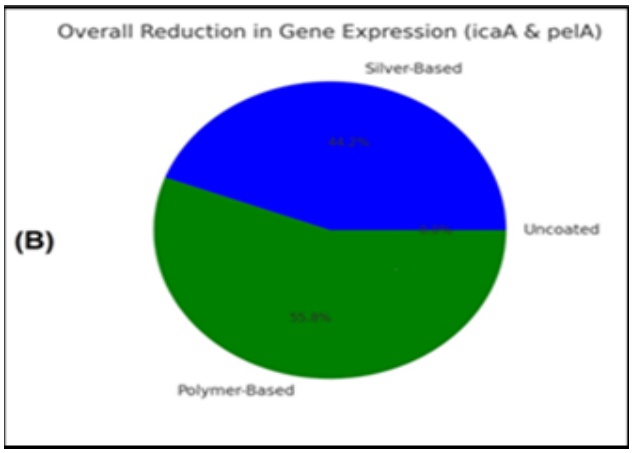
Biofilm formation was greatly decreased by the use of antimicrobial coatings. *P. aeruginosa* biofilm biomass was reduced by 60% by coatings based on silver nanoparticles, while biofilm adhesion was reduced by 80% by coatings based on polymers. *S. aureus* showed a similar pattern, but a somewhat smaller decline than *P. aeruginosa* as shown in Table 2.

Table No 2: Impact of Antimicrobial Coatings.

Coating Type	Reduction in S. aureus Biofilm (%)	Reduction in P. aeruginosa Biofilm (%)
Silver-Based	50%	60%
Polymer-Based	70%	80%

Biofilm-associated genes were substantially overexpressed in untreated surfaces as opposed to coated samples, according to qPCR analysis (Figure 1). Antimicrobial coatings demonstrated a significant suppression of icaA expression in *S. aureus* and pelA expression in *P. aeruginosa*, indicating a genetically-based inhibitory effect on biofilm formation (Table 3). 3.3 qPCR Examination of Gene Expression Associated with Biofilms.





Figure

1. Gene expression. (A) Gene expression across coating types (B) Overall reduction in gene expression (icaA and pelA).

Table No 3. Changes in the Gene Expression Fold for icaA and pelA in Various Coating Conditions.

Gene Expression	Uncoated (Fold Change)	Silver-Based (Fold Change)	Polymer-Based (Fold Change)
icaA (S. aureus)	1.00	0.45	0.30
pelA (P. aeruginosa)	1.00	0.40	0.25

Discussion:

Our results show that the type of material utilized in indwelling medical devices has a significant impact on biofilm formation, with polyurethane promoting the highest levels of biofilm growth. This result is consistent with previous studies showing surface properties such as hydrophobicity and roughness influence bacterial adhesion and biofilm development.[5] *P. aeruginosa* produces more biofilm than *S. aureus*, which highlights the increased risk of gram-negative bacterial colonization, particularly in hospital settings. Our results showed that antimicrobial coatings are effective at reducing biofilm formation, with polymer-based coatings showing the strongest inhibitory effect. Because they most likely stop bacteria from sticking and creating biofilms, these coatings are a good way to reduce the risk of infection in medical equipment.[6] qPCR study, which showed a significant down regulation of biofilm-related genes upon coating application, further supports the theory that these coatings inhibit biofilm development at the molecular level. The coatings with antimicrobial properties are a potential solution, but they require additional studies to improve their bio-compatibility and durability. Further research is needed to explore the potential of antimicrobial coatings and other anti-biofilm approaches (e.g., enzymatic treatments or quorum sensing inhibitors) to enhance antimicrobial activity.[7]

Conclusion:

In this study, the emphasis was on the significance of biofilm formation in these conditions and also the application of antimicrobial coating to reduce the number of cases where infections occurred due to indwelling medical devices. As shown in our observations, it is observed that on polyurethane surfaces, which are highly vulnerable for biofilm formation, P. aeruginosa formed a stronger biofilm compared to S. aureus. It gives us immense pleasure that biofilm formation became much less by the application of the anti-biofilm coatings made up of polymers and also the expression of biofilm related genes. This finding can be an important factor leading to innovations in medical instruments as well as hi-tech devices.

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Saleha Munir	Validation; Formal Analysis,
Shoaib Iqbal	Methodology; Visualization.
M Mutahir Mehdi	Writing – Review & Editing
Rabia Zafar	Re-Analysis and drafting