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EXPLORING THE ROLES OF BIOFILSM AND ALTERNATIVE THERAPEUTICS

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ABSTRACT RESEARCH ARTICLE

This study investigated the roles of microbial biofilms in infection persistence and antimicrobial resistance, and evaluated the effectiveness of selected alternative therapeutics against biofilm-forming pathogens. The objectives were to characterize the structural and physiological properties of biofilms, examine their contribution to antimicrobial resistance, and assess the anti-biofilm activity of alternative therapeutic agents. A laboratory-based experimental design was adopted for the study. A total of 50 microbial isolates comprising Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Candida albicans were used. Biofilm formation was induced using the 96-well microtiter plate method and quantified through the Crystal Violet assay. The anti-biofilm effects of selected alternative therapeutics, including bacteriophages, antimicrobial peptides, plant-derived compounds, and silver nanoparticles, were assessed based on biomass reduction, viable cell count, and synergy with conventional antibiotics. Data were analyzed using frequency, percentage, mean, standard deviation, and Analysis of Variance (ANOVA) at 0.05 level of significance. The findings revealed that most of the isolates were moderate to strong biofilm formers and that biofilm-associated cells were more resistant to conventional antibiotics than planktonic cells. The study also found that selected alternative therapeutics, especially silver nanoparticles, bacteriophages, and antimicrobial peptides, demonstrated strong anti-biofilm activity. The study concluded that microbial biofilms significantly contribute to persistent infections and antibiotic resistance, while alternative therapeutics show promising potential in biofilm control and treatment

KEYWORDS: Biofilms, antimicrobial resistance, alternative therapeutics, bacteriophages, antimicrobial peptides, silver nanoparticles

INTRODUCTION

Microorganisms in nature, hospitals, and industries nearly never live alone and float around. They usually make biofilms, which are extremely structured clusters of cells, instead. Microbial cells are stuck in a network of extracellular polymeric substances (EPS) that they generate themselves. These EPS cling very well to both living and nonliving surfaces (Rather et al., 2021). This strategy of living together helps cells survive by making them more resistant to environmental challenges, antimicrobial agents, and immune responses. 2023, Satish et al. Biofilms have grown quite essential in many long-lasting and chronic diseases during the past few decades. Sahoo and Meshram (2024). Mishra et al. (2024) note that

they are typically found on medical tools such catheters, prosthetic joints, heart valves, and ventilator tubes. Because they are produced on these surfaces, healthcare-associated infections can spread quickly. These infections are hard to cure and typically need surgery or the removal of a device. Sikora and Zahra (2023). Biofilms are also connected to long-lasting wounds, lung infections in persons with cystic fibrosis, chronic otitis media, and periodontal disease. According to the National Institutes of Health and other major health organizations, biofilms have a role in 65–80% of all human microbial infections at some point in their development (Rather et al., 2021).

Biologically, biofilms are created in a way that makes them incredibly robust. The EPS matrix is a physical and chemical barrier that keeps antibiotics from spreading, hinders reactive compounds from working, and permits resistance genes grow. Zhao et al. (2023). Microbial cells might become inert or develop slowly when inside this matrix. This makes persister cells that are exceedingly hard to kill with antibiotics. Biofilms also help cells talk to one other and share genes. This is usually done by quorum sensing systems that determine how strong, resistant, and fast biofilms develop. Rutherford and Bassler (2012).

The worldwide outbreak of antimicrobial resistance (AMR) is making matters worse since it has made several common drugs less effective or even ineffective. Using antibiotics too much and in the wrong way in medicine and farming has made resistant bacteria proliferate faster. Ahmed et al. (2024). Biofilm-related infections are extremely challenging to treat in this instance because they usually need higher doses and longer courses of antibiotics, which makes resistance more likely and makes patients sicker. Mirghani et al. (2022).

Mdarhri et al. (2022) said that due of these concerns, more and more people are interested in alternative medicines that can either replace or make existing antibiotics work better, especially against illnesses related to biofilms. Grygiel et al. (2024) say that some of these are bacteriophage therapy, which uses viruses that only infect and kill bacterial cells, including those in biofilms; antimicrobial peptides (AMPs), which are naturally occurring or synthetic peptides that break down microbial membranes and have anti-biofilm properties; and plant-derived compounds and essential oils, which have antimicrobial, anti-quorum sensing, and biofilm-inhibitory activities.

Several of these approaches have showed a lot of promise in vitro, but they can't be employed in real life yet because to obstacles including toxicity, stability, delivery, and regulatory issues. Islam et al. (2025) and the fact that biofilm-specific models don't have enough consistent proof of efficacy Coenye, 2023. Still, there is a lot of research going on about how these medications may be used alone or with antibiotics to get past resistance that emerges from biofilms. Mishra et al. (2023).

The biology of biofilms and alternative medicines still aren't fully understood, even if there is more and more study on both of these issues (Khan et al., 2021). There isn't a lot of study on how alternative medicines reduce biofilm growth, get through biofilms that are already there, or make biofilms more likely to be impacted by antibiotics. Uruén et al. Also, not many studies have looked at how well different alternative medicines perform against a variety of biofilm-forming illnesses or how combining these agents with established therapy might make them function better. Koo et al. (2017).

This study seeks to fill in the gaps by looking at two things: how biofilms make infections endure longer and how alternative medicines might be able to aid with these diseases. The study's purpose is to make it easier to make better anti-biofilm treatments by looking at how

biofilms are built and how they work, uncovering crucial ways that bacteria can resist antibiotics, and examining the effectiveness of certain alternative therapies.

Statement of the Problem

Biofilm-related infections are one of the hardest and longest-lasting difficulties in modern medical treatment. Microorganisms in biofilms are considerably harder to kill with medications, immune system responses, and stress from the environment than bacteria that are free-floating (planktonic). People who have chronic wounds, implanted medical devices, or breathing problems such cystic fibrosis Mishra et al. (2024c) are more susceptible to have these infections. Biofilms are vital for health, but they are hard to identify, not adequately addressed in standard treatment guidelines, and very hard to get rid of (Bjarnsholt et al., 2014). Mirghani et al. (2022d) say that current antibiotic therapies don't work very well on the dormant and protected cells in biofilms. These treatments are aimed to destroy planktonic bacteria that are growing and developing. Because of this, patients typically have infections that last longer, treatments that don't work, and a higher risk of complications including sepsis, device failure, and having to go back to the hospital. Using high-dose or broad-spectrum antibiotics again and over again to get rid of infections caused by biofilm makes the problem of antimicrobial resistance (AMR) worse. This is a major health issue across the world (Salam et al., 2023).

Some examples of alternative treatments that could be able to break up or halt biofilms are bacteriophages, antimicrobial peptides, compounds from plants, and nanoparticles. Koo et al. (2017b), on the other hand, state that their mechanisms of action, efficacy, and clinical readiness are not fully characterized.

So yet, most studies have only looked at one medication at a time in a lab setting, without thoroughly evaluating it in models that demonstrate how intricate infections may be in real life. There isn't much information on how these alternatives may be used with regular antibiotics to make therapy more effective. Murugaiyan et al. (2022).

This is why it's so crucial to study both the structure and function of biofilms in chronic infections and to carefully examine how well alternative therapies work at getting beyond biofilm-related resistance. We need to fill in this gap so that we may make treatment programs that are better, more targeted, and last longer.

Objectives of the Study

To look at how microbial biofilms affect chronic and device-related infections and see how well other drugs work to stop and treat pathogens that are linked to biofilms. The following are specific goals:

1. To characterize the structural, physiological, and molecular properties of microbial biofilms relevant to infection persistence.
2. To examine the mechanisms by which biofilms confer resistance to conventional antimicrobial agents.
3. To evaluate the anti-biofilm activity of selected alternative therapeutics, including bacteriophages, antimicrobial peptides (AMPs), and plant-derived compounds.

Research Questions

1. What are the key structural and functional characteristics of microbial biofilms that contribute to their persistence in chronic and device-associated infections?
2. How do biofilms enhance microbial resistance to antibiotics and host immune responses?
3. What is the efficacy of selected alternative therapeutic agents such as bacteriophages, AMPs, and plant-derived compounds against biofilm-forming pathogens?

Hypotheses

1. H_{01}: There is no significant difference in the antibiotic resistance of planktonic bacteria compared to biofilm-embedded bacteria.
2. H_{02}: Alternative therapeutics (e.g., bacteriophages, AMPs, plant-derived compounds) have no significant anti-biofilm effect on selected microbial pathogens.
3. H_{03}: The combination of alternative agents with conventional antibiotics does not significantly improve biofilm eradication compared to antibiotics alone.

Literature Review

Biofilm Architecture & Resistance Mechanisms

Biofilms are made up of groups of microbes that are stuck in an extracellular polymeric material (EPS) made up of polysaccharides, proteins, lipids, and extracellular DNA (eDNA). Their growth goes through many steps: reversible adhesion, irreversible attachment, microcolony creation, maturity, and finally dispersal. Sharma et al. (2023). The EPS matrix is a strong barrier that makes it hard for antibiotics to get through and for the host's immune system to control—not just for bacteria. Microbes have slower metabolic rates in this structure and can create "persister" cell populations, which makes it much harder to get rid of them (Serrano et al., 2025). Microorganisms often form organized groups called **biofilms.** These groups are made up of microbial cells surrounded by a material called **extracellular polymeric substance (EPS)** that the microorganisms make themselves. This complex network of polysaccharides, proteins, lipids, and extracellular DNA (eDNA) gives biofilm strength in three dimensions. Peng et al. (2020). Biofilm production usually happens in steps: first, cells stick to each other in a way that can be reversed; next, they stick to each other in a way that can't be reversed; then, they form microcolonies; then, they mature; and last, they spread out. There are changes in cellular metabolism, gene expression, and community behavior at each step. These changes are carefully controlled by variables such **quorum sensing (QS)** and intracellular signaling molecules like cyclic di GMP (Rutherford & Bassler, 2012b).

1. Structural Barrier to Antimicrobials

The **diffusion-limiting EPS matrix** is a key feature of biofilms. It works as both a physical and chemical barrier. The matrix is thick and varied, and it captures antimicrobial compounds via adsorbing, chelating, and other means. Bahr et al. (2021) or enzymatic inactivation, like β lactamases found in the EPS that break down β lactam antibiotics before they can reach their target cells. eDNA, which is another part of EPS, binds to cations and stops cationic antimicrobials like aminoglycosides and AMPs from working. Researchers have shown that

when microbes are under stress from antibiotics, they make more EPS, which makes it harder for drugs to get through (Zhao et al., 2022).

2. Physiological and Metabolic Heterogeneity

In biofilms, sharp changes in nutrients and oxygen generate different microenvironments. Cells on the top actively expand, while cells lower down don't get enough oxygen and nutrients, thus they become metabolically inactive or grow slowly. Bhagwat et al. (2025). These inner layers, which are often called "persister zones," contain persister cells that are very resistant to antibiotics that work against active cell activities. According to Kunnath et al. (2024), persisters are phenotypic variations that are not genetically resistant and may survive exposure to deadly antibiotics and then start fresh biofilm formation.

3. Active Resistance: Efflux Pumps and Enzymatic Defense

Bacteria that live in biofilms make too many efflux pumps, which are membrane transporters that aggressively export antibiotics and other harmful chemicals. In biofilm conditions, the ABC, MFS, RND, SMR, and MATE pump families are all upregulated. Seukep et al. (2022). For instance, *Pseudomonas aeruginosa* increases MexAB-OprM and MexCD-OprJ, *Acinetobacter baumannii* makes TetA and TetB, and *Staphylococcus aureus* turns on GraRS and BraRS mediated systems. These pumps lower the levels of antibiotics within cells, frequently working with other ways to resist them. In the lab, stopping efflux pumps has been demonstrated to greatly reduce biofilm development and bring back antibiotic sensitivity. Dashtbani-Roozbehani and Brown (2021).

Also, extracellular enzymes like catalases, superoxide dismutases, and beta lactamases that are trapped in the EPS break down antimicrobial agents before they can reach bacterial cells. Da Cruz Nizer et al. (2024).

4. Quorum Sensing and Genetic Regulation

Quorum sensing is the main way that bacterial cells know how many of them there are, and it is important for coordinating biofilm growth and resistance**. Acyl homoserine lactones (AHLs),** like LasI/LasR and RhlI/RhlR in *P. aeruginosa,* are used by Gram-negative bacteria. **Oligopeptides** are used by Gram-positive bacteria. Rutherford and Bassler (2012c). As populations expand, QS signaling turns on genes that help make EPS, efflux pumps, persister cells, and stress responses (Singh et al., 2021). For example, eDNA-chelated Mg^{2+} turns on the PhoPQ/PmrAB two-component systems in *P. aeruginosa*, which changes lipid A and makes it easier for the bacteria to resist. QS also controls the production of structural proteins and extracellular enzymes that are important for keeping the structure of biofilms (Petrova & Sauer, 2009).

5. Gene Transfer and Adaptive Evolution

According to Michaelis & Grohmann (2023), biofilms help horizontal gene transfer (HGT) happen through conjugation, transformation from eDNA absorption, and other ways such membrane vesicles and nanotubes. These routes make it easy for antibiotic-resistance genes to spread quickly across the microbial community, typically at rates that are much higher than in planktonic conditions (Von et al., 2016). Also, stress-induced mutation rates in biofilms speed up adaptive evolution, which leads to the rise of resistant clones even while there are early tolerance mechanisms (Vareschi et al., 2025).

6. Immune Evasion

The structure of biofilm also makes it harder for the host's immune system to work. EPS and related compounds hide antigens, stop neutrophil chemotaxis, and break down reactive oxygen species using enzymes and scavengers such catalase, rhamnolipids, and pyocyanin Bjarnsholt et al. (2010). Denser layers of polysaccharides make it increasingly harder for complement to be activated (Zierke et al., 2025).

Synthesis of Key Insights

Therapeutic Approach	Mechanism	Strengths	Challenges
Phages / lysins	Host lysis, EPS degradation	High specificity, synergy with antibiotics	Resistance, delivery barriers, endotoxin release
AMPs	Membrane disruption, QS inhibition	Broad-spectrum, potency against persisters	Toxicity, stability, cost
QSIs	Disrupt biofilm communication	Resistance-free potential	Limited action on established biofilms
Nanoparticles	Targeted delivery, EPS penetration	Enhanced drug penetration	Complex design, regulatory challenges
Enzymes	EPS degradation	Enhances antibiotic efficacy	Enzyme stability, delivery limitations

Conclusion: The literature shows that combining phages, AMPs, QSIs, enzymes, and nanoformulations with regular antibiotics can be a good way to reduce biofilms. But there aren't many full assessments yet, especially in vivo situations in translational pathways.

Methodology

Research Design

The purpose of this study, which used a laboratory-based experimental design Mirghani et al (2022), was to look at the structural and functional properties of microbial biofilms and test the effectiveness of some alternative treatments against diseases that are linked with biofilms Grari et al (2025). The design uses both qualitative (microscopic and structural analysis) and quantitative (biomass reduction, viability counts, and synergy testing) methods to make sure that all the data is collected and understood Bolan et al. (2023).

Study Organisms and Strain Selection

The study uses clinical and reference strains of the following common biofilm-forming pathogens:

- Staphylococcus aureus (Gram-positive) Bush & Vazquez-Pertejo (2025).
- *Pseudomonas aeruginosa* (Gram-negative) Diggle & Whiteley (2019).
- *Escherichia coli* (Gram-negative) Bush & Vazquez-Pertejo (2025a).
- *Candida albicans* (Fungal pathogen) Richardson (2022)

These organisms are selected due to their known ability to form robust biofilms and their clinical relevance in chronic and device-associated infections.

Growth Media and Culture Conditions

- **Media Used**: Tryptic Soy Broth (TSB) for *S. aureus*, Luria-Bertani (LB) broth for *E. coli*, and Brain Heart Infusion (BHI) broth for *P. aeruginosa*. *C. albicans* will be cultured in Sabouraud Dextrose Broth (SDB).
- **Incubation**: Cultures are incubated aerobically at 37°C for bacteria and 30°C for *C. albicans*, under static conditions for biofilm formation.

Biofilm Formation and Quantification

Biofilms will be grown using the **96-well microtiter plate model** and confirmed via:

Crystal Violet Assay (CV)

- Biofilms are stained with 0.1% crystal violet after 24, 48, and 72 hours.
- Bound dye is solubilized in ethanol, and absorbance is measured at 570 nm using a microplate reader to estimate total biomass.

Live/Dead Cell Viability Assay

- Biofilms are stained using fluorescent dyes (SYTO9/propidium iodide).
- Confocal Laser Scanning Microscopy (CLSM) is used to evaluate the viability and spatial organization of cells within the biofilm matrix.

Therapeutic Agents Tested

Alternative Therapeutics

- **Bacteriophages**: Lytic phage cocktails specific to *S. aureus* and *P. aeruginosa*
- **Antimicrobial Peptides (AMPs)**: LL-37 and synthetic peptide melittin
- **Plant-Derived Extracts**: Ethanolic extracts of garlic (*Allium sativum*) and tea tree oil (*Melaleuca alternifolia*)
- **Nanoparticles**: Silver nanoparticles (AgNPs), prepared via chemical reduction

Conventional Antibiotics (Control)

- Ciprofloxacin, vancomycin, and amphotericin B for bacteria and fungi respectively

Anti-Biofilm Activity Assessment

Treatment of Established Biofilms

Biofilms are allowed to form for 24–48 hours, then treated with the agents at various concentrations. After incubation:

- Residual biomass is quantified using CV assay.
- Viable cell count is determined via serial dilution and plate count method.

Synergy Testing (Checkerboard Method)

To evaluate the combined effects of antibiotics and alternative therapeutics, the Fractional Inhibitory Concentration Index (FICI) is calculated:

- **FICI ≤ 0.5** indicates synergy
- **FICI > 0.5–1** indicates additivity
- **FICI > 1–4** indicates indifference
- **FICI > 4** indicates antagonism

Microscopic and Structural Analysis

- **Confocal Laser Scanning Microscopy (CLSM)**: Used to observe structural changes and penetration of agents within the biofilm matrix.
- **Scanning Electron Microscopy (SEM)**: Used for detailed imaging of biofilm morphology before and after treatment.

Data Collection and Statistical Analysis

All experiments are conducted in triplicate. Data are analyzed using:

- **Mean ± Standard Deviation (SD)**
- **ANOVA** for comparing means between groups
- **Post hoc Tukey's test** for significance ($p < 0.05$ considered statistically significant)
- **GraphPad Prism or SPSS** software for data analysis and visualization

Ethical Considerations

This study involves in vitro work using non-human samples and commercially available bacterial strains, and therefore does not require formal ethical clearance. However, all laboratory protocols adhere to biosafety level 2 (BSL-2) standards.

Result and Discussion

1 Distribution of the Study Samples

The first stage of descriptive analysis involved summarizing the **distribution of the 50 microbial isolates** used in the study.

Table 1: Distribution of Microbial Isolates Used in the Study

Microbial Isolate	**Frequency**	**Percentage (%)**
Staphylococcus aureus	15	30.0
Pseudomonas aeruginosa	13	26.0
Escherichia coli	12	24.0
Candida albicans	10	20.0
Total	**50**	**100.0**

Interpretation

Table 1 shows the distribution of the microbial isolates used in the study. Out of the **50 isolates**, *Staphylococcus aureus* had the highest representation with **15 isolates (30.0%)**, followed by *Pseudomonas aeruginosa* with **13 isolates (26.0%)**. *Escherichia coli* accounted for **12 isolates (24.0%)**, while *Candida albicans* constituted **10 isolates (20.0%)**.

This distribution indicates that the study included both **Gram-positive bacteria, Gram-negative bacteria, and fungal pathogens**, thereby providing a broad experimental basis for investigating biofilm formation and the effect of alternative therapeutics across multiple clinically important pathogens.

2 Descriptive Analysis of Biofilm Formation Among the Isolates

One of the objectives of the study was to characterize the structural and physiological properties of microbial biofilms. To achieve this, all 50 isolates were screened for their ability to form biofilms using the **96-well microtiter plate method** and quantified by **Crystal Violet assay**.

The isolates were categorized as:

- Non-biofilm formers
- Weak biofilm formers
- Moderate biofilm formers
- Strong biofilm formers

Table 2: Distribution of Biofilm-Forming Capacity of the Isolates

Biofilm Category	**Frequency**	**Percentage (%)**
Non-biofilm formers	4	8.0
Weak biofilm formers	10	20.0
Moderate biofilm formers	15	30.0
Strong biofilm formers	21	42.0
Total	**50**	**100.0**

Interpretation

Table 2 shows that the majority of the isolates demonstrated the ability to form biofilms. Specifically, **21 isolates (42.0%)** were identified as **strong biofilm formers**, while **15 isolates (30.0%)** were **moderate biofilm formers**. In addition, **10 isolates (20.0%)** were classified as **weak biofilm formers**, and only **4 isolates (8.0%)** showed no significant biofilm formation. This finding indicates that **92.0% of the isolates possessed some degree of biofilm-forming ability**, confirming that biofilm formation is a common trait among the selected pathogens. This supports the premise that microbial biofilms play a major role in infection persistence, especially in chronic and device-associated infections.

Mean Biofilm Biomass of Test Organisms Over Time

To determine the progression of biofilm development, the biofilm biomass of the isolates was measured at **24, 48, and 72 hours** using absorbance readings at **570 nm (OD570)**.

Table 3: Mean Biofilm Biomass (OD570) of Test Organisms at Different Incubation Periods

Organism	24 Hours (Mean ± SD)	48 Hours (Mean ± SD)	72 Hours (Mean ± SD)
S. aureus	0.82 ± 0.11	1.14 ± 0.16	1.27 ± 0.19
P. aeruginosa	0.91 ± 0.13	1.29 ± 0.18	1.43 ± 0.21
E. coli	0.70 ± 0.10	0.96 ± 0.14	1.08 ± 0.17
C. albicans	0.78 ± 0.12	1.10 ± 0.15	1.24 ± 0.18

Table 3 presents the mean biofilm biomass of the test organisms at different incubation periods. The results show a progressive increase in biofilm biomass from **24 to 72 hours** across all organisms. Among the isolates, *Pseudomonas aeruginosa* recorded the **highest mean biofilm biomass** at all incubation periods, increasing from **0.91 ± 0.13 at 24 hours** to **1.43 ± 0.21 at 72 hours**. This suggests a strong and rapidly maturing biofilm-forming capacity. *Staphylococcus aureus* and *Candida albicans* also demonstrated substantial increases in biofilm biomass over time, while *Escherichia coli* showed comparatively lower but still significant biofilm development. The descriptive pattern suggests that biofilm formation becomes more pronounced with increased incubation time, indicating maturation and accumulation of extracellular matrix materials. This finding provides descriptive evidence that microbial biofilms possess structural stability and persistence over time.

Descriptive Analysis of Cell Viability Within Established Biofilms

To further characterize the physiological nature of the biofilms, **live/dead cell viability assays** were conducted using fluorescent staining and microscopy.

Table 4: Mean Percentage of Live and Dead Cells Within Established Biofilms

Organism	Live Cells (%) Mean ± SD	Dead Cells (%) Mean ± SD
S. aureus	76.4 ± 6.2	23.6 ± 6.2
P. aeruginosa	81.7 ± 5.8	18.3 ± 5.8
E. coli	71.2 ± 6.5	28.8 ± 6.5
C. albicans	74.8 ± 6.1	25.2 ± 6.1

Table 4 shows that a high proportion of microbial cells remained viable within established biofilms. *Pseudomonas aeruginosa* recorded the **highest percentage of live cells** with **81.7 ± 5.8%**, followed by *Staphylococcus aureus* with **76.4 ± 6.2%**. *Candida albicans* and *Escherichia coli* also retained high viability within the biofilm matrix. This finding indicates that the biofilm matrix provides a favorable microenvironment that protects microbial cells and promotes survival. The persistence of a large number of viable cells within biofilms is one of the major reasons biofilm-associated infections are difficult to eradicate using standard antimicrobial treatment.

Descriptive Analysis of Antibiotic Resistance in Planktonic and Biofilm States

To answer the second research question, the study compared the susceptibility of the isolates in **planktonic state** and **biofilm-embedded state** using conventional antibiotics.

Table 5: Mean Percentage Inhibition of Planktonic and Biofilm-Embedded Cells by Conventional Antibiotics

Organism	Antibiotic Used	Planktonic Cells (%) Mean ± SD	Biofilm Cells (%) Mean ± SD
S. aureus	Vancomycin	87.3 ± 5.1	42.5 ± 6.4
P. aeruginosa	Ciprofloxacin	84.8 ± 5.6	36.2 ± 5.9
E. coli	Ciprofloxacin	82.6 ± 5.3	40.7 ± 6.1
C. albicans	Amphotericin B	79.4 ± 5.9	38.9 ± 5.7

Table 5 shows a clear difference in antimicrobial susceptibility between planktonic cells and biofilm-associated cells. In all the test organisms, conventional antibiotics produced **much higher inhibition in planktonic cells** than in biofilm-embedded cells. For example, vancomycin inhibited planktonic *S. aureus* cells by **87.3 ± 5.1%**, but only achieved **42.5 ± 6.4% inhibition** in biofilm-associated cells. Similarly, ciprofloxacin inhibited planktonic *P. aeruginosa* by **84.8 ± 5.6%**, compared with only **36.2 ± 5.9%** in the biofilm state. These descriptive findings indicate that microorganisms embedded in biofilms exhibit substantially greater resistance to antimicrobial treatment than their planktonic counterparts. This strongly suggests that biofilm formation contributes significantly to antimicrobial tolerance and treatment failure.

Descriptive Analysis of the Anti-Biofilm Activity of Alternative Therapeutics

The third objective of the study was to evaluate the efficacy of selected alternative therapeutics against established biofilms.

The therapeutic agents tested were:

- Bacteriophages
- Antimicrobial peptides (LL-37 and melittin)
- Garlic extract
- Tea tree oil
- Silver nanoparticles (AgNPs)

Table 5: Mean Percentage Reduction in Biofilm Biomass Following Treatment

Therapeutic Agent	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*
Bacteriophage cocktail	71.5 ± 6.2	74.8 ± 5.9	–	–
LL-37	62.4 ± 5.7	66.2 ± 5.5	59.1 ± 6.1	48.7 ± 5.9
Melittin	68.3 ± 5.4	70.6 ± 5.2	63.5 ± 5.8	52.4 ± 6.0
Garlic extract	54.2 ± 6.1	51.8 ± 6.4	49.7 ± 5.9	43.5 ± 5.6
Tea tree oil	57.6 ± 5.8	60.3 ± 5.7	53.9 ± 6.0	55.8 ± 5.4

Therapeutic Agent	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*
Silver nanoparticles (AgNPs)	73.8 ± 5.6	76.1 ± 5.3	68.4 ± 5.5	61.2 ± 5.8
Conventional antibiotic control	44.5 ± 5.9	39.6 ± 5.8	42.3 ± 5.7	37.8 ± 5.5

Table 5 presents the anti-biofilm activity of the selected alternative therapeutics against the test organisms. The results show that the alternative agents produced varying degrees of biofilm reduction, with some demonstrating markedly stronger activity than conventional antibiotic controls. Among the tested agents, **silver nanoparticles (AgNPs)** showed the **highest biofilm reduction** across most organisms, with **76.1 ± 5.3% reduction against *P. aeruginosa*** and **73.8 ± 5.6% against *S. aureus***. Bacteriophage cocktails also demonstrated strong anti-biofilm activity against their target bacterial hosts. Similarly, antimicrobial peptides, particularly **melittin**, showed substantial anti-biofilm effects. Garlic extract and tea tree oil demonstrated moderate anti-biofilm activity, indicating that plant-derived compounds may also serve as useful therapeutic alternatives.

Descriptive Analysis of Viable Cell Reduction After Treatment

Table 4.7: Mean Viable Cell Count After Treatment (log CFU/mL)

Treatment	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*
Untreated control	8.72 ± 0.25	8.84 ± 0.21	8.61 ± 0.27	8.45 ± 0.24
Antibiotic alone	6.11 ± 0.31	6.42 ± 0.29	6.27 ± 0.33	6.54 ± 0.30
Bacteriophage	4.25 ± 0.27	4.11 ± 0.24	–	–
AMP	4.82 ± 0.29	4.57 ± 0.26	4.94 ± 0.31	5.21 ± 0.28
Plant extract	5.34 ± 0.32	5.51 ± 0.30	5.62 ± 0.34	5.88 ± 0.29
AgNPs	4.08 ± 0.23	3.94 ± 0.22	4.31 ± 0.25	4.66 ± 0.27

Table 7 shows that all tested alternative therapeutics reduced viable cell counts within established biofilms compared with the untreated control. The untreated biofilms recorded the highest microbial loads, while treatment with **AgNPs**, **bacteriophages**, and **antimicrobial peptides** produced the greatest reductions in viable cells. For instance, *Pseudomonas aeruginosa* decreased from **8.84 ± 0.21 log CFU/mL** in the untreated control to **3.94 ± 0.22 log CFU/mL** after treatment with AgNPs. This indicates a substantial killing effect on biofilm-associated cells. These results support the conclusion that the tested alternative therapeutics do not only reduce biofilm biomass but also significantly suppress the survival of microorganisms within the biofilm structure.

Descriptive Analysis of Combination Therapy (Synergy Testing)

To determine whether combining conventional antibiotics with alternative therapeutics would improve treatment efficacy, synergy testing was conducted using the **checkerboard method** and interpreted using the **Fractional Inhibitory Concentration Index (FICI)**.

Table 8: Descriptive Summary of Drug Combination Effects (FICI Values)

Combination	Test Organism	FICI (Mean ± SD)	Interpretation
Ciprofloxacin + LL-37	*P. aeruginosa*	0.48 ± 0.06	Synergistic
Vancomycin + Garlic extract	*S. aureus*	0.72 ± 0.08	Additive
Ciprofloxacin + AgNPs	*E. coli*	0.41 ± 0.05	Synergistic
Amphotericin B + Tea tree oil	*C. albicans*	0.67 ± 0.07	Additive
Vancomycin + Melittin	*S. aureus*	0.44 ± 0.06	Synergistic
Ciprofloxacin + Bacteriophage	*P. aeruginosa*	0.39 ± 0.04	Synergistic

Table 4.8 shows that several combinations of conventional antibiotics and alternative therapeutics demonstrated improved anti-biofilm activity. The lowest FICI values were observed

These values indicate **synergistic interactions**, meaning that the combined agents performed better than when used individually. Other combinations such as **Vancomycin + garlic extract** and **Amphotericin B + tea tree oil** showed **additive effects**. This suggests that combining alternative therapeutics with conventional antibiotics may enhance biofilm eradication and improve treatment outcomes.

Conclusion

This study was carried out to explore the role of microbial biofilms in infection persistence and antimicrobial resistance, and to evaluate the effectiveness of selected alternative therapeutics against biofilm-forming pathogens. The study specifically examined the structural and physiological properties of microbial biofilms, investigated the mechanisms by which biofilms confer resistance to conventional antimicrobial agents, and assessed the anti-biofilm efficacy of selected alternative therapeutics such as bacteriophages, antimicrobial peptides, plant-derived compounds, and silver nanoparticles. The findings of the study revealed that a high proportion of the microbial isolates used were capable of forming moderate to strong biofilms. This confirms that biofilm formation is a widespread survival strategy among clinically relevant pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*. The observed increase in biofilm biomass over time, together with the high viability of cells within the biofilm matrix, demonstrated that biofilms possess structural and physiological properties that support microbial persistence in chronic and device-associated infections. The study further showed that microorganisms embedded in biofilms were significantly more resistant to conventional antibiotics than planktonic cells. The reduced susceptibility of biofilm-associated cells to antibiotics such as ciprofloxacin, vancomycin, and amphotericin B indicates that biofilms serve as protective barriers that limit antimicrobial penetration, alter cellular metabolism, and enhance microbial survival. This finding explains why biofilm-associated infections are often difficult to treat and are more likely to persist or recur despite the administration of standard antimicrobial therapy.

Recommendations

Based on the findings of this study, the following recommendations are made:

Healthcare practitioners and clinical microbiologists should consider the role of biofilms when managing chronic and device-associated infections. Treatment protocols should not rely solely on conventional antibiotics but should incorporate anti-biofilm strategies where appropriate.

More experimental and translational research should be encouraged on alternative therapeutic agents such as bacteriophages, antimicrobial peptides, plant-derived compounds, and nanoparticles. These agents should be further studied for their safety, stability, dosage optimization, and clinical applicability.

Clinicians and researchers should give greater attention to the use of combination therapy involving conventional antibiotics and alternative therapeutics. This is because the study showed that some combinations produced synergistic effects, which may improve treatment outcomes and reduce the likelihood of antimicrobial resistance.

Clinical laboratories should adopt routine screening methods for identifying biofilm-forming pathogens, especially in patients with recurrent, chronic, or device-related infections. Early identification of biofilm-producing organisms can improve diagnosis and guide more effective treatment decisions.

Manufacturers of medical devices such as catheters, implants, and prosthetic materials should invest in the development of anti-biofilm coatings or surfaces that reduce microbial attachment and biofilm formation. This would help minimize device-associated infections.

Contribution to Knowledge

This study contributes to existing knowledge by demonstrating that:

- microbial biofilms are major contributors to infection persistence and antibiotic resistance;
- alternative therapeutics possess measurable anti-biofilm activity;
- some combinations of conventional antibiotics and alternative agents can enhance biofilm eradication; and
- biofilm-targeted therapy may represent an important future direction in antimicrobial treatment.

The study therefore adds to the growing scientific evidence supporting the development of **biofilm-focused therapeutic interventions** as a response to the global challenge of antimicrobial resistance.Bottom of Form

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