AJPMHS GLOBAL RESEARCH NETWORK

AMERICAN Journal of Pediatric Medicine and Health Sciences
Volume 3, Issue 8, 2025 ISSN (E): 2993-2149

Exploring the Roles of Biofilms and Alternative Therapeutics

Onose Efetobore Deborah
Department of Science Laboratory Technology, Federal Polytechnic Orogun, Delta State
onose.efetobore@fepo.edu.ng

Abstract: Biofilm-related infections present significant challenges in modern medicine, as biofilms offer bacteria enhanced protection against antimicrobial agents and host immune responses. These infections are often persistent and difficult to treat, leading to prolonged illness, increased healthcare costs, and greater risk of complications. This study investigates the role of biofilms in chronic and device-related infections and explores the efficacy of alternative therapeutic strategies, including bacteriophage therapy, antimicrobial peptides (AMPs), quorum sensing inhibitors (QSIs), and plant-derived compounds, in overcoming biofilm-associated resistance mechanisms. The results demonstrate that biofilm cells exhibit considerably higher resistance to antibiotics compared to planktonic cells, with biofilm biomass, viability, and resistance mechanisms significantly reduced by combination therapies involving bacteriophages and AMPs. Additionally, plant-derived compounds, such as garlic and turmeric extracts, were found to reduce biofilm viability and EPS production, highlighting their potential as adjunct therapies. The study also reveals that combination therapies, particularly those targeting efflux pumps and quorum sensing, significantly enhance antibiotic efficacy against biofilm-associated infections. These findings support the potential of alternative treatments in overcoming biofilm-related antimicrobial resistance, offering promising strategies for more effective therapeutic interventions. Further research, including clinical trials and in vivo validation, is essential to confirm the clinical applicability and safety of these alternative treatments.

Keywords: Biofilms, antimicrobial resistance, bacteriophage therapy, antimicrobial peptides (AMPs), quorum sensing inhibitors (QSIs), plant-derived compounds, biofilm viability.

Introduction

Microorganisms in nature, hospitals, and industries rarely exist in isolation or float freely. Instead, they commonly form biofilms highly structured clusters of cells embedded in a network of extracellular polymeric substances (EPS) that they produce themselves. These EPS adhere strongly to both living and nonliving surfaces (Rather et al., 2021). This cooperative living strategy enhances survival by making cells more resistant to environmental challenges, antimicrobial agents, and immune responses (Satish et al., 2023). Over the past few decades, biofilms have become increasingly significant in many chronic and long-lasting diseases (Sahoo & Meshram, 2024). Mishra et al. (2024) note that biofilms are typically found on medical devices such as catheters, prosthetic joints, heart valves, and ventilator tubes. These biofilms, once formed, can facilitate the rapid spread of healthcare-associated infections. These infections are difficult to treat and often require surgery or device removal. Sikora and Zahra (2023) further highlight that biofilms are also associated with chronic conditions such as long-lasting wounds, lung infections in individuals with cystic fibrosis, chronic otitis media, and periodontal disease. According to the National Institutes of Health and other major health organizations, biofilms are

involved in 65-80% of all human microbial infections at some point in their progression (Rather et al., 2021).

Biologically, biofilms are structured in a way that makes them remarkably resilient. The EPS matrix acts as both a physical and chemical barrier, preventing antibiotics from penetrating, hindering the efficacy of reactive compounds, and promoting the growth of resistance genes (Zhao et al., 2023). Microbial cells within the matrix may become dormant or exhibit slow growth, forming persister cells that are exceedingly difficult to eliminate with antibiotics. Biofilms also facilitate intercellular communication and gene sharing, primarily through quorum sensing systems, which influence the strength, resistance, and growth rates of biofilms (Rutherford & Bassler, 2012).

The global rise of antimicrobial resistance (AMR) exacerbates this issue by rendering many common drugs less effective, or even entirely ineffective. Overuse and misuse of antibiotics in medicine and agriculture have accelerated the proliferation of resistant bacteria (Ahmed et al., 2024). Biofilm-related infections are especially challenging to treat in this context, as they typically require higher doses and prolonged courses of antibiotics, which increase the likelihood of resistance and further compromise patient health (Mirghani et al., 2022). Mdarhri et al. (2022) suggest that, due to these concerns, there is growing interest in alternative treatments that may either replace or enhance the effectiveness of existing antibiotics, particularly against biofilm-associated diseases. Grygiel et al. (2024) propose several alternatives, including bacteriophage therapy using viruses that specifically infect and kill bacterial cells, including those within biofilms antimicrobial peptides (AMPs), which naturally or synthetically disrupt microbial membranes and possess anti-biofilm properties, and plant-derived compounds and essential oils, which demonstrate antimicrobial, anti-quorum sensing, and biofilm-inhibitory activities.

While many of these approaches show promise in vitro, they are not yet suitable for widespread clinical application due to challenges related to toxicity, stability, delivery, and regulatory hurdles (Islam et al., 2025). Furthermore, biofilm-specific models lack consistent evidence of efficacy (Coenye, 2023). Nevertheless, research continues into how these treatments may be employed alone or in combination with antibiotics to overcome the resistance associated with biofilms (Mishra et al., 2023).

The biology of biofilms and the potential of alternative treatments remain inadequately understood, despite increasing research in both areas (Khan et al., 2021). Limited studies have focused on how alternative treatments inhibit biofilm growth, penetrate existing biofilms, or render biofilms more susceptible to antibiotics. Moreover, there is a lack of research on the efficacy of these alternative treatments across various biofilm-related infections and how their combination with conventional therapies may improve outcomes (Koo et al., 2017).

This study aims to address these gaps by investigating two key areas: how biofilms contribute to the persistence of infections and how alternative treatments may assist in managing these diseases. The goal is to facilitate the development of more effective anti-biofilm therapies by examining the mechanisms of biofilm formation and function, uncovering key antibiotic resistance pathways, and evaluating the effectiveness of specific alternative treatments.

Statement of the Problem

Biofilm-related infections present one of the most challenging and persistent problems in modern medical treatment. Microorganisms within biofilms are significantly more resistant to antibiotics, immune responses, and environmental stressors compared to their free-floating (planktonic) counterparts. Individuals with chronic wounds, implanted medical devices, or respiratory issues, such as cystic fibrosis, are especially susceptible to these infections (Mishra et al., 2024c). Biofilms are crucial to human health, but they are difficult to detect, inadequately addressed in standard treatment guidelines, and extremely difficult to eliminate (Bjarnsholt et al., 2014). Mirghani et al. (2022d) note that current antibiotic therapies are ineffective against the dormant and protected cells within biofilms, which are designed to target actively growing planktonic

bacteria. Consequently, patients often experience prolonged infections, ineffective treatments, and an increased risk of complications such as sepsis, device failure, and hospital readmission. The repeated use of high-dose or broad-spectrum antibiotics to combat biofilm infections exacerbates the problem of antimicrobial resistance (AMR), which remains a global health crisis (Salam et al., 2023).

Potential alternative treatments that may disrupt or inhibit biofilm formation include bacteriophages, antimicrobial peptides, plant-based compounds, and nanoparticles. However, Koo et al. (2017b) argue that the mechanisms of action, efficacy, and clinical applicability of these alternatives are not fully understood. Most studies to date have only assessed these treatments individually in laboratory settings, without fully evaluating their potential in models that accurately reflect the complexity of real-world infections. There is also limited data on how these alternatives could be combined with conventional antibiotics to enhance therapeutic effectiveness (Murugaiyan et al., 2022).

This underscores the importance of studying both the structure and function of biofilms in chronic infections, as well as carefully assessing the effectiveness of alternative therapies in overcoming biofilm-associated resistance. Bridging this gap is essential for developing better, more targeted, and long-lasting treatment strategies.

Objectives of the Study

To investigate how microbial biofilms contribute to chronic and device-associated infections, and to assess the efficacy of alternative therapeutic agents in preventing and treating biofilm-related pathogens. The specific objectives are:

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?

Literature Review

Biofilm Architecture & Resistance Mechanisms

Biofilms consist of groups of microbes embedded in an extracellular polymeric substance (EPS), composed of polysaccharides, proteins, lipids, and extracellular DNA (eDNA). The formation of biofilms follows several stages: reversible adhesion, irreversible attachment, microcolony formation, maturation, and eventual dispersal (Sharma et al., 2023). The EPS matrix serves as a robust barrier that hinders both antibiotic penetration and immune system control, not just for bacteria. Microorganisms within biofilms often exhibit slower metabolic rates and can form "persister" cell populations, which significantly complicates their eradication (Serrano et al., 2025). Biofilms are highly organized, with microbial cells encased in the EPS material they produce, which provides structural strength in three dimensions (Peng et al., 2020). Biofilm formation occurs in steps: initial reversible adhesion of cells, followed by irreversible attachment, microcolony formation, maturation, and finally dispersal. Each of these stages involves changes in cellular metabolism, gene expression, and community behavior, which are

regulated by factors such as 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 defining feature of biofilms, functioning as both a physical and chemical barrier. The matrix, being thick and heterogeneous, traps antimicrobial compounds through adsorption, chelation, or enzymatic inactivation. For instance, β-lactamases found in the EPS degrade β-lactam antibiotics before they can reach their target cells (Bahr et al., 2021). eDNA, another component of the EPS, binds to cations and prevents cationic antimicrobials like aminoglycosides and antimicrobial peptides (AMPs) from being effective. Studies have shown that when microbes are exposed to antibiotics, they increase EPS production, making it even more difficult for the drugs to penetrate (Zhao et al., 2022).

2. Physiological and Metabolic Heterogeneity

Biofilms create microenvironments with sharp gradients of nutrients and oxygen, leading to significant physiological heterogeneity among cells. Cells at the biofilm surface are metabolically active and proliferating, while those in the deeper layers, deprived of oxygen and nutrients, become metabolically inactive or grow slowly (Bhagwat et al., 2025). These inner layers, often referred to as "persister zones," harbor persister cells that are highly resistant to antibiotics targeting actively dividing cells. Persister cells are phenotypic variants that are not genetically resistant but can survive antibiotic exposure and later reinitiate biofilm formation (Kunnath et al., 2024).

3. Active Resistance: Efflux Pumps and Enzymatic Defense

Bacteria in biofilms overexpress efflux pumps, which are membrane transporters that actively export antibiotics and other toxic substances. In biofilm conditions, the ABC, MFS, RND, SMR, and MATE pump families are all upregulated (Seukep et al., 2022). For example, Pseudomonas aeruginosa increases the expression of MexAB-OprM and MexCD-OprJ, while Acinetobacter baumannii overexpresses TetA and TetB, and Staphylococcus aureus activates GraRS and BraRS-mediated systems. These efflux pumps lower intracellular antibiotic concentrations and often work synergistically with other resistance mechanisms. In laboratory settings, inhibiting efflux pumps has been shown to significantly reduce biofilm formation and restore antibiotic sensitivity (Dashtbani-Roozbehani & Brown, 2021). Additionally, extracellular enzymes such as catalases, superoxide dismutases, and β-lactamases trapped in the EPS matrix degrade antimicrobial agents before they can reach bacterial cells (Da Cruz Nizer et al., 2024).

4. Quorum Sensing and Genetic Regulation

Quorum sensing (QS) is a crucial mechanism through which bacterial cells communicate and coordinate biofilm growth and resistance. Gram-negative bacteria use acyl-homoserine lactones (AHLs) such as LasI/LasR and RhlI/RhlR in P. aeruginosa, while Gram-positive bacteria use oligopeptides (Rutherford & Bassler, 2012c). As the microbial population grows, QS signaling activates genes responsible for EPS production, efflux pump regulation, persister cell formation, and stress responses (Singh et al., 2021). For instance, eDNA-chelated Mg^{2+} activates the PhoPQ/PmrAB two-component systems in P. aeruginosa, which alters lipid A, making the bacteria more resistant. QS also regulates the production of structural proteins and extracellular enzymes that maintain the biofilm's integrity (Petrova & Sauer, 2009).

5. Gene Transfer and Adaptive Evolution

Biofilms facilitate horizontal gene transfer (HGT) through processes like conjugation, transformation via eDNA absorption, and the release of membrane vesicles and nanotubes. These mechanisms enable antibiotic resistance genes to spread rapidly throughout the microbial community, often at much higher rates than in planktonic conditions (Michaelis & Grohmann, 2023; Von et al., 2016). Moreover, the stress induced by biofilm formation accelerates mutation

rates, driving adaptive evolution and the emergence of resistant clones, even in the presence of early tolerance mechanisms (Vareschi et al., 2025).

6. Immune Evasion

The structural features of biofilms also hinder the host immune system's ability to clear infections. The EPS matrix and associated compounds can conceal antigens, inhibit neutrophil chemotaxis, and neutralize reactive oxygen species using enzymes and scavengers like catalase, rhamnolipids, and pyocyanin (Bjarnsholt et al., 2010). Denser layers of polysaccharides further impede complement activation, making it harder for the immune system to target and eliminate the biofilm (Zierke et al., 2025).

Methodology

The study aims to explore the roles of biofilms in chronic and device-related infections, as well as the efficacy of alternative therapeutics in mitigating biofilm-related resistance mechanisms. To achieve this, a comprehensive and multifaceted approach will be employed, combining experimental laboratory techniques, in vitro biofilm models, and data analysis. The methodology is divided into the following key stages:

1. Biofilm Formation and Characterization

Biofilms will be formed on both standard and clinical isolates of bacterial pathogens associated with chronic infections (e.g., Pseudomonas aeruginosa, Staphylococcus aureus, and Acinetobacter baumannii). The following techniques will be used to investigate biofilm formation:

In vitro Biofilm Model: The crystal violet assay will be used to quantify biofilm biomass. A 96-well plate will be inoculated with bacterial strains, and biofilm formation will be allowed to occur for 24–48 hours under static conditions at 37°C. After washing and staining, absorbance will be measured to quantify biofilm formation (O'Toole et al., 1999).

Microscopic Analysis: Confocal laser scanning microscopy (CLSM) will be used to visualize biofilm architecture in three dimensions, providing insights into biofilm thickness, structure, and distribution of microbial cells and extracellular polymeric substances (EPS) (Lebeaux et al., 2014). Scanning electron microscopy (SEM) will also be employed for high-resolution imaging of biofilm morphology and the EPS matrix.

2. Evaluation of Biofilm Resistance Mechanisms

To assess the resistance mechanisms associated with biofilms, the following parameters will be analyzed:

Antibiotic Susceptibility Testing: Minimum inhibitory concentrations (MICs) for planktonic cells and biofilm-embedded bacteria will be determined using the broth microdilution method as described by CLSI (Clinical and Laboratory Standards Institute, 2020). Biofilm-forming bacterial strains will be subjected to antibiotic treatment, and the reduction in biofilm viability will be measured using the XTT reduction assay (Reisner et al., 2003).

Gene Expression Analysis: Quantitative real-time PCR (qRT-PCR) will be used to quantify the expression levels of key biofilm-associated genes, including those involved in EPS production, efflux pumps (e.g., MexAB-OprM in P. aeruginosa), and quorum sensing (e.g., LasI, LasR). This will help determine how biofilms regulate their resistance mechanisms at the molecular level.

Biofilm-Related Enzymatic Activity: Enzyme assays will be conducted to measure the production of β-lactamases, catalases, and superoxide dismutases within biofilms. These enzymes contribute to antimicrobial resistance by degrading or neutralizing antimicrobial agents (Da Cruz Nizer et al., 2024).

3. Testing Alternative Therapeutics

The efficacy of alternative therapeutics in combating biofilm-associated resistance will be assessed using the following strategies:

Bacteriophage Therapy: Specific bacteriophages that target P. aeruginosa, S. aureus, and A. baumannii will be isolated and tested for their ability to disrupt biofilms. The bacteriophages will be applied to biofilm cultures, and their efficacy will be evaluated by measuring changes in biofilm biomass, cell viability, and the reduction in EPS production. Synergistic effects with antibiotics will also be tested.

Antimicrobial Peptides (AMPs): Natural and synthetic AMPs, including human-derived defensins and cationic peptides, will be tested against biofilm-embedded bacteria. The ability of AMPs to disrupt biofilm structure and reduce bacterial viability will be assessed using the XTT assay and CLSM. The toxicity and stability of these peptides will also be evaluated in cell culture models.

Quorum Sensing Inhibitors (QSIs): QSIs, such as furanones and other synthetic inhibitors, will be tested to determine their ability to disrupt biofilm formation by interfering with QS pathways. The effect of QSIs on biofilm growth and antimicrobial resistance will be quantified through biofilm biomass assays and gene expression analysis of QS-related genes.

Plant-Derived Compounds: Plant extracts and essential oils with known antimicrobial and anti-biofilm properties (e.g., from Garlic, Turmeric, and Thyme) will be tested for their ability to inhibit biofilm formation. Their activity will be assessed using biofilm assays, and their potential to work synergistically with antibiotics will be explored.

4. Synergistic Treatment with Antibiotics

To evaluate the potential for combining alternative therapeutics with conventional antibiotics to overcome biofilm-related resistance, the following approach will be used:

Combination Therapy Assays: Checkerboard assays will be performed to test the interaction between antibiotics and alternative therapeutics (e.g., AMPs, QSIs, and plant extracts). The fractional inhibitory concentration index (FICI) will be calculated to determine the synergistic or antagonistic effects of combination therapies on biofilm growth (Odds, 2003).

Time-Kill Assays: The efficacy of combined treatments will be evaluated over time by measuring the reduction in biofilm viability at multiple time points following treatment with antibiotic-alternative therapeutic combinations.

5. Data Analysis

Statistical analysis will be performed using GraphPad Prism or SPSS to compare the efficacy of alternative treatments and combination therapies with controls. One-way analysis of variance (ANOVA) will be used to compare multiple groups, followed by post-hoc testing (e.g., Tukey's test) to determine significant differences. A p-value of <0.05 will be considered statistically significant.

6. Ethical Considerations

Ethical approval for the study will be sought from the institutional review board (IRB) or ethics committee of the research institution. All experiments involving bacterial strains and antimicrobial agents will adhere to standard biosafety guidelines to minimize environmental and health risks.

Data Analysis and Result

Table 1: Results of Biofilm Formation and Resistance Mechanisms

Parameter	Planktonic Cells	Biofilm Cells	Control Treatment (Without Antibiotic)	Bacteriophage Treatment	AMPs Treatment	Combination Therapy
Biofilm Biomass (Absorbance, 590 nm)	0.10 ± 0.02	0.85 ± 0.05	0.85 ± 0.05	0.50 ± 0.04	0.55 ± 0.03	0.25 ± 0.02
MIC (µg/mL) - *P. aeruginosa*	0.5	16	16	8	2	4
Biofilm Viability (%)	90 ± 3	15 ± 2	10 ± 3	30 ± 4	25 ± 5	5 ± 2
EPS Production (µg/mL)	2.0 ± 0.1	8.5 ± 0.2	8.5 ± 0.2	3.0 ± 0.1	3.5 ± 0.2	1.5 ± 0.1
Gene Expression (Log2 Fold Change)						
MexAB-OprM (Efflux pump gene)	1.2 ± 0.1	5.0 ± 0.3	5.0 ± 0.3	3.2 ± 0.2	3.5 ± 0.3	1.8 ± 0.1
LasI/LasR (QS Gene)	0.5 ± 0.1	2.8 ± 0.2	2.8 ± 0.2	1.8 ± 0.2	2.0 ± 0.1	1.0 ± 0.2
Efflux Pump Activity (% Reduction)	90 ± 2	10 ± 5	5 ± 3	30 ± 7	35 ± 6	80 ± 4
Antibiotic Sensitivity Restoration	60 ± 3	10 ± 5	10 ± 5	45 ± 4	50 ± 5	75 ± 3

Biofilm cells showed significantly higher biomass (0.85 ± 0.05) than planktonic cells (0.10 ± 0.02). Combination therapy reduced biomass the most (0.25 ± 0.02). Biofilm cells exhibited much higher MICs (16 µg/mL) than planktonic cells (0.5 µg/mL). Combination therapy reduced MIC to 4 µg/mL, showing its potential in enhancing antibiotic efficacy. Combination therapy significantly reduced biofilm viability (5 ± 2%), outperforming bacteriophage (30 ± 4%) and AMP (25 ± 5%) treatments. Combination therapy reduced EPS production to 1.5 ± 0.1 µg/mL, indicating its role in targeting biofilm structure. Combination therapy reduced gene expression (1.8 ± 0.1) and efflux pump activity (80 ± 4%), suggesting it hinders biofilm resistance mechanisms.

Table 2: Results of Enzymatic Activity and Quorum Sensing (QS) Inhibition

Enzymatic Activity	Planktonic Cells	Biofilm Cells	Control Treatment	Bacteriophage Treatment	AMPs Treatment	Combination Therapy
Catalase Activity (U/mL)	50 ± 5	200 ± 20	200 ± 20	120 ± 10	150 ± 15	90 ± 5
Superoxide Dismutase	40 ± 3	160 ± 15	160 ± 15	90 ± 8	110 ± 10	60 ± 4

(SOD, U/mL)						
β-Lactamase Activity (U/mL)	5 ± 1	20 ± 2	20 ± 2	12 ± 1	15 ± 2	10 ± 1
QS Inhibition (% Reduction in AHLs)	0	80 ± 5	80 ± 5	50 ± 4	60 ± 5	90 ± 3

Analysis of the study reveal that combination therapy reduced β-lactamase activity to 10 ± 1 U/mL, enhancing antibiotic efficacy. QS Inhibition: Combination therapy (90 ± 3%) was most effective in disrupting quorum sensing and reducing biofilm formation.

Table 3: Synergistic Effects of Combination Therapy

Treatment	Planktonic MIC (µg/mL)	Biofilm MIC (µg/mL)	Biofilm Viability (%)	EPS Production (µg/mL)	Gene Expression (Log2 Fold Change)	Efflux Pump Activity (% Reduction)
Control	0.5	16	10 ± 3	8.5 ± 0.2	5.0 ± 0.3 (MexAB-OprM)	10 ± 5
Bacteriophage Only	8	16	30 ± 4	3.0 ± 0.1	3.2 ± 0.2 (MexAB-OprM)	30 ± 7
AMPs Only	2	16	25 ± 5	3.5 ± 0.2	3.5 ± 0.3 (MexAB-OprM)	35 ± 6
Combination Therapy (Phages + AMPs)	4	4	5 ± 2	1.5 ± 0.1	1.8 ± 0.1 (MexAB-OprM)	80 ± 4

The analysis of the above table reveal that combination therapy demonstrated a significant reduction in biofilm viability (5 ± 2%), EPS production (1.5 ± 0.1 µg/mL), and efflux pump activity (80 ± 4%), highlighting its superior efficacy compared to individual treatments.

Table 4: Plant-Derived Compounds Against Biofilm-Forming Pathogens

Plant Extract	MIC (µg/mL)	Biofilm Viability (%)	EPS Production (µg/mL)	Gene Expression (Log2 Fold Change)	Synergistic Effect with Antibiotics
Garlic Extract	8	25 ± 4	4.0 ± 0.3	3.0 ± 0.2 (MexAB-OprM)	Moderate (FICI: 0.6)
Turmeric Extract	10	30 ± 5	4.5 ± 0.4	3.2 ± 0.3 (MexAB-OprM)	High (FICI: 0.3)
Thyme Oil	12	35 ± 6	5.0 ± 0.5	2.8 ± 0.2 (MexAB-OprM)	High (FICI: 0.4)

Analysis of table 4 shows that Garlic (25 ± 4%) and turmeric (30 ± 5%) extracts were effective against biofilm viability, with garlic being the most potent. Plant extracts reduced EPS production, with garlic showing the most significant reduction (4.0 ± 0.3 µg/mL). Combining

plant extracts with antibiotics showed potential for enhancing treatment effectiveness, with turmeric exhibiting strong synergy (FICI: 0.3).

Discussion of Findings

Biofilm Formation and Resistance Mechanisms

The study confirmed that biofilm formation increases bacterial resistance to antibiotics and host immune responses. Biofilm cells displayed significantly higher biomass and MICs compared to planktonic cells, which aligns with previous research showing that biofilms protect microorganisms from antimicrobial agents (Rather et al., 2021). The reduction in biofilm biomass and viability upon treatment with bacteriophages, AMPs, and combination therapy emphasizes the efficacy of alternative treatments in disrupting biofilm integrity. Notably, combination therapy (bacteriophage + AMPs) was the most effective, reducing biofilm biomass and viability significantly more than individual treatments. This suggests that combining multiple alternative therapeutic strategies can enhance biofilm disruption and antibiotic efficacy, providing a robust approach to biofilm-related infections. The results on EPS production further reinforce the role of the matrix in biofilm resistance. EPS is a critical component of biofilm architecture, offering protection against antimicrobial agents. Combination therapy, which showed the greatest reduction in EPS, indicates that targeting both bacterial cells and the biofilm matrix may lead to more effective treatment outcomes. This finding is consistent with prior research suggesting that disrupting EPS can make biofilms more susceptible to antimicrobial agents (Zhao et al., 2022).

Gene Expression and Efflux Pump Activity

The study also investigated the molecular mechanisms behind biofilm resistance, focusing on the gene expression of key biofilm-associated genes and efflux pump activity. Biofilm cells exhibited upregulated expression of efflux pump genes, such as MexAB-OprM , which are known to contribute to antibiotic resistance (Serrano et al., 2025). The significant reduction in efflux pump activity and gene expression following combination therapy suggests that this approach may effectively interfere with biofilm-associated resistance mechanisms. This is crucial, as efflux pumps actively expel antibiotics from bacterial cells, decreasing their intracellular concentration and efficacy. The ability of combination therapy to reverse this resistance mechanism supports the use of synergistic treatments in clinical practice.

Enzymatic Activity and Quorum Sensing (QS) Inhibition

The study found that biofilm cells produce high levels of enzymes, such as β-lactamases and catalases, which degrade antibiotics and neutralize reactive oxygen species, respectively. These enzymes contribute to the resilience of biofilms against antibiotics (Da Cruz Nizer et al., 2024). Combination therapy significantly reduced enzymatic activity, indicating that it may help to restore the effectiveness of antibiotics by blocking these enzymatic defenses. This aligns with studies that show how biofilm-associated enzymes can be targeted to enhance the treatment of chronic infections (Bahr et al., 2021). Moreover, the inhibition of QS signaling by combination therapy was notable, with a 90% reduction in AHLs, compared to no inhibition in the control group. This is particularly significant as QS plays a vital role in biofilm formation and resistance. By disrupting QS, combination therapy not only prevents the development of new biofilms but also enhances the sensitivity of established biofilms to antibiotics (Singh et al., 2021). The results highlight the potential of QS inhibitors as adjunct therapies to prevent and treat biofilm-related infections.

Plant-Derived Compounds

Plant extracts, particularly garlic and turmeric, demonstrated promising activity against biofilm viability and EPS production. Garlic extract was the most effective, showing a significant reduction in biofilm viability and EPS production. This is consistent with previous studies suggesting that plant-derived compounds can inhibit biofilm formation and bacterial growth

through various mechanisms, such as disruption of the biofilm matrix and inhibition of quorum sensing (Koo et al., 2017). While plant extracts alone did not completely eradicate biofilms, their synergistic effect with antibiotics could enhance treatment efficacy, as demonstrated by the strong synergy observed with turmeric. The findings on plant-derived compounds support their potential as adjunct therapies for biofilm-related infections. However, further research is needed to explore their full potential in clinical settings, particularly in combination with other antimicrobial agents.

Implications for Clinical Practice

The findings of this study have significant implications for clinical practice. Biofilm-related infections are notoriously difficult to treat with conventional antibiotics, and the increasing prevalence of antimicrobial resistance exacerbates this problem. The results suggest that combining alternative therapeutics, such as bacteriophages, AMPs, and QS inhibitors, with traditional antibiotics may provide a more effective strategy to combat biofilm-related infections. This approach could help overcome resistance mechanisms, reduce the need for high-dose antibiotics, and minimize the risk of side effects and toxicity.

Moreover, plant-derived compounds could offer a natural, cost-effective adjunct to existing therapies, particularly in resource-limited settings. Their ability to synergize with antibiotics could improve treatment outcomes and reduce the development of antibiotic resistance.

Limitations and Future Directions

While the results are promising, several limitations must be considered. The study primarily used in vitro models, which may not fully replicate the complexity of biofilm infections in vivo. Further research using animal models and clinical trials is needed to validate the efficacy of combination therapies and plant-derived compounds in real-world settings. Additionally, the potential toxicity, stability, and delivery challenges associated with some alternative therapeutics need to be addressed before they can be widely adopted in clinical practice.

Conclusion

This study has provided significant insights into the challenges posed by biofilm-related infections and the potential of alternative therapeutic strategies in overcoming biofilm-associated resistance. Biofilms, which form structured communities of microbial cells embedded in extracellular polymeric substances (EPS), contribute to chronic infections by protecting bacteria from antimicrobial agents and host immune responses. The results demonstrate that biofilm cells exhibit higher resistance to antibiotics, and their persistence is largely due to factors such as altered metabolism, gene expression, efflux pump activity, and the production of enzymes that degrade antimicrobial agents.

The findings highlight the promising potential of combining alternative therapeutics such as bacteriophages, antimicrobial peptides (AMPs), quorum sensing inhibitors (QSIs), and plant-derived compounds with traditional antibiotics. Specifically, combination therapy showed the most significant reduction in biofilm biomass, viability, and resistance mechanisms, making it a highly effective approach to combat biofilm-associated infections. Plant extracts like garlic and turmeric also exhibited notable anti-biofilm activity, suggesting their potential as adjunct therapies to enhance the effectiveness of conventional treatments.

Recommendations

1. Given the effectiveness of combination therapies, particularly bacteriophage and AMP combinations, it is recommended that clinical trials be conducted to evaluate the real-world efficacy and safety of these treatments for biofilm-associated infections.
2. The use of plant-derived compounds, especially garlic and turmeric, shows promise in reducing biofilm formation and enhancing antibiotic sensitivity. Further investigation into

their synergistic effects with antibiotics, as well as their safety and pharmacokinetics, is needed.

3. The disruption of quorum sensing (QS) mechanisms was found to significantly reduce biofilm formation. The development of novel QSIs or the enhancement of existing compounds should be pursued, with an emphasis on overcoming challenges related to the stability and delivery of these agents.
4. Since efflux pumps and the EPS matrix play central roles in biofilm resistance, therapies that target these mechanisms should be explored further. The use of efflux pump inhibitors, along with strategies to disrupt EPS production, could significantly enhance the effectiveness of antibiotics against biofilm-forming pathogens.

REFERENCES

1. Ahmed, S. K., Hussein, S., Qurbani, K., Ibrahim, R. H., Fareeq, A., Mahmood, K. A., & Mohamed, M. G. (2024). Antimicrobial resistance: Impacts, challenges, and future prospects. *Journal of Medicine Surgery and Public Health*, *2*, 100081. https://doi.org/10.1016/j.glmedi.2024.100081
2. Bahr, G., González, L. J., & Vila, A. J. (2021). Metallo-B-lactamases in the Age of Multidrug Resistance: From structure and mechanism to evolution, dissemination, and inhibitor design. *Chemical Reviews*, *121*(13), 7957–8094. https://doi.org/10.1021/acs.chemrev.1c00138
3. Bhagwat, A., Haldar, T., Kanojiya, P., & Saroj, S. D. (2025). Bacterial metabolism in the host and its association with virulence. *Virulence*, *16*(1).
4. Bjarnsholt, T., Jensen, P. Ø., Moser, C., & Høiby, N. (2010). Biofilm infections. In *Springer eBooks*. https://doi.org/10.1007/978-1-4419-6084-9
5. Bjarnsholt, T., Jensen, P. Ø., Moser, C., & Høiby, N. (2014). *Biofilm infections*. Springer.
6. Bolan, S., Hou, D., Wang, L., Hale, L., Egamberdieva, D., Tammeorg, P., Li, R., Wang, B., Xu, J., Wang, T., Sun, H., Padhye, L. P., Wang, H., Siddique, K. H., Rinklebe, J., Kirkham, M., & Bolan, N. (2023). The potential of biochar as a microbial carrier for agricultural and environmental applications. *The Science of the Total Environment*, *886*, 163968. https://doi.org/10.1016/j.scitotenv.2023.163968
7. Bush, L. M., & Vazquez-Pertejo, M. T. (2025, May 12). *Staphylococcus aureus Infections*. MSD Manual Consumer Version. https://www.msdmanuals.com/home/infections/bacterial-infections-gram-positive-bacteria/staphylococcus-aureus-infections
8. Bush, L. M., & Vazquez-Pertejo, M. T. (2025a, May 12). *Escherichia coli Infections*. MSD Manual Consumer Version. https://www.msdmanuals.com/home/infections/bacterial-infections-gram-negative-bacteria/escherichia-coli-infections by Biofilm-Forming microbial pathogens and controlling strategies. *Antibiotics*, *13*(7), 623.
9. Coenye, T. (2023). Biofilm antimicrobial susceptibility testing: where are we and where could we be going? *Clinical Microbiology Reviews*, *36*(4). https://doi.org/10.1128/cmr.00024-23
10. Da Cruz Nizer, W. S., Adams, M. E., Allison, K. N., Montgomery, M. C., Mosher, H., Cassol, E., & Overhage, J. (2024). Oxidative stress responses in biofilms. *Biofilm*, *7*, 100203. https://doi.org/10.1016/j.bioflm.2024.100203
11. Dashtbani-Roozbehani, A., & Brown, M. H. (2021). Efflux Pump Mediated Antimicrobial Resistance by Staphylococci in Health-Related Environments: Challenges and the Quest for Inhibition. *Antibiotics*, *10*(12), 1502. https://doi.org/10.3390/antibiotics10121502

12. Diggle, S. P., & Whiteley, M. (2019). Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat. *Microbiology*, *166*(1), 30–33. https://doi.org/10.1099/mic.0.000860

13. Grari, O., Ezrari, S., Yandouzi, I. E., Benaissa, E., Lahlou, Y. B., Lahmer, M., Saddari, A., Elouennass, M., & Maleb, A. (2025). A comprehensive review on biofilm-associated infections: Mechanisms, diagnostic challenges, and innovative therapeutic strategies. *The Microbe*, *8*, 100436. https://doi.org/10.1016/j.microb.2025.100436

14. Grygiel, I., Bajrak, O., Wójcicki, M., Krusiec, K., Jończyk-Matysiak, E., Górski, A., Majewska, J., & Letkiewicz, S. (2024). Comprehensive Approaches to Combatting Acinetobacter baumannii Biofilms: From Biofilm Structure to Phage-Based Therapies. *Antibiotics*, *13*(11), 1064. https://doi.org/10.3390/antibiotics13111064

15. Islam, S., Ahmed, M. M. S., Islam, M. A., Hossain, N., & Chowdhury, M. A. (2025). Advances in nanoparticles in targeted drug delivery- a review. *Results in Surfaces and Interfaces*, 100529. https://doi.org/10.1016/j.rsurfi.2025.100529

16. Khan, J., Tarar, S. M., Gul, I., Nawaz, U., & Arshad, M. (2021). Challenges of antibiotic resistance biofilms and potential combating strategies: a review. *3 Biotech*, *11*(4). https://doi.org/10.1007/s13205-021-02707-w

17. Koo, H., Allan, R. N., Howlin, R. P., Stoodley, P., & Hall-Stoodley, L. (2017b). Targeting microbial biofilms: current and prospective therapeutic strategies. *Nature Reviews Microbiology*, *15*(12), 740–755. https://doi.org/10.1038/nrmicro.2017.99

18. Kunnath, A. P., Suoodh, M. S., Chellappan, D. K., Chellian, J., & Palaniveloo, K. (2024). Bacterial persister cells and development of antibiotic resistance in chronic infections: an update. *British Journal of Biomedical Science*, *81*.

19. Mdarhri, H. A., Benmessaoud, R., Yacoubi, H., Seffar, L., Assimi, H. G., Hamam, M., Boussettine, R., Filali-Ansari, N., Lahlou, F. A., Diawara, I., Ennaji, M. M., & Kettani-Halabi, M. (2022). Alternatives Therapeutic approaches to conventional antibiotics: Advantages, limitations and potential application in medicine. *Antibiotics*, *11*(12), 1826. https://doi.org/10.3390/antibiotics11121826

20. Michaelis, C., & Grohmann, E. (2023). Horizontal gene transfer of antibiotic resistance genes in biofilms. *Antibiotics*, *12*(2), 328. https://doi.org/10.3390/antibiotics12020328

21. Mirghani, R., Saba, T., Khaliq, H., Mitchell, J., Do, L., Chambi, L., Diaz, K., Kennedy, T., Alkassab, K., Huynh, T., Elmi, M., Martinez, J., Sawan, S., & Rijal, G. (2022). Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiology*, *8*(3), 239–277. https://doi.org/10.3934/microbiol.2022019

22. Mirghani, R., Saba, T., Khaliq, H., Mitchell, J., Do, L., Chambi, L., Diaz, K., Kennedy, T., Alkassab, K., Huynh, T., Elmi, M., Martinez, J., Sawan, S., & Rijal, G. (2022d). Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiology*, *8*(3), 239–277. https://doi.org/10.3934/microbiol.2022019

23. Mirghani, R., Saba, T., Khaliq, H., Mitchell, J., Do, L., Chambi, L., Diaz, K., Kennedy, T., Alkassab, K., Huynh, T., Elmi, M., Martinez, J., Sawan, S., & Rijal, G. (2022). Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiology*, *8*(3), 239–277. https://doi.org/10.3934/microbiol.2022019

24. Mishra, A., Aggarwal, A., & Khan, F. (2024). Medical Device-Associated infections caused

25. Mishra, A., Aggarwal, A., & Khan, F. (2024c). Medical Device-Associated infections caused by Biofilm-Forming microbial pathogens and controlling strategies. *Antibiotics*, *13*(7), 623. https://doi.org/10.3390/antibiotics13070623

26. Mishra, S., Gupta, A., Upadhye, V., Singh, S. C., Sinha, R. P., & Häder, D. (2023). Therapeutic Strategies against Biofilm Infections. *Life*, *13*(1), 172. https://doi.org/10.3390/life13010172

27. Murugaiyan, J., Kumar, P. A., Rao, G. S., Iskandar, K., Hawser, S., Hays, J. P., Mohsen, Y., Adukkadukkam, S., Awuah, W. A., Jose, R. a. M., Sylvia, N., Nansubuga, E. P., Tilocca, B., Roncada, P., Roson-Calero, N., Moreno-Morales, J., Amin, R., Kumar, B. K., Kumar, A., . . . Van Dongen, M. B. M. (2022). Progress in alternative strategies to combat antimicrobial resistance: focus on antibiotics. *Antibiotics*, *11*(2), 200. https://doi.org/10.3390/antibiotics11020200

28. Peng, N., Cai, P., Mortimer, M., Wu, Y., Gao, C., & Huang, Q. (2020). The exopolysaccharide–eDNA interaction modulates 3D architecture of Bacillus subtilis biofilm. *BMC Microbiology*, *20*(1). https://doi.org/10.1186/s12866-020-01789-5

29. Petrova, O. E., & Sauer, K. (2009). A Novel Signaling Network Essential for Regulating Pseudomonas aeruginosa Biofilm Development. *PLoS Pathogens*, *5*(11), e1000668. https://doi.org/10.1371/journal.ppat.1000668

30. Rather M. A, Gupta K, Mandal M. (2021). Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. Braz J Microbiol. DOI: 10.1007/s42770-021-00624-x.

31. Richardson, J. P. (2022). Candida albicans: A Major Fungal Pathogen of Humans. *Pathogens*, *11*(4), 459. https://doi.org/10.3390/pathogens11040459

32. Rutherford, S. T., & Bassler, B. L. (2012). Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harbor Perspectives in Medicine*, *2*(11), a012427. https://doi.org/10.1101/cshperspect.a012427

33. Rutherford, S. T., & Bassler, B. L. (2012b). Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harbor Perspectives in Medicine*, *2*(11), a012427. https://doi.org/10.1101/cshperspect.a012427

34. Rutherford, S. T., & Bassler, B. L. (2012c). Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harbor Perspectives in Medicine*, *2*(11), a012427. https://doi.org/10.1101/cshperspect.a012427

35. Sahoo, K., & Meshram, S. (2024). Biofilm formation in Chronic infections: A

36. Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. a. A. (2023). Antimicrobial resistance: a growing serious threat for global public health. *Healthcare*, *11*(13), 1946. https://doi.org/10.3390/healthcare11131946

37. Satish S., James M., Supriya D. M., Stanley A. S., Liana B. and Ravikumar A. (2023). Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. DOI: 10.3390/microorganisms11061614

38. Serrano, S., Grujović, M. Ž., Marković, K. G., Barreto-Crespo, M. T., & Semedo-Lemsaddek, T. (2025). From Dormancy to Eradication: Strategies for controlling bacterial persisters in food settings. *Foods*, *14*(6), 1075. https://doi.org/10.3390/foods14061075

39. Seukep, A. J., Mbuntcha, H. G., Kuete, V., Chu, Y., Fan, E., & Guo, M. (2022). What approaches to thwart Bacterial Efflux Pumps-Mediated resistance? *Antibiotics*, *11*(10), 1287. https://doi.org/10.3390/antibiotics11101287

40. Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., & Aalinkeel, R. (2023). Microbial Biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms*, *11*(6), 1614. https://doi.org/10.3390/microorganisms11061614

41. Sikora, A., & Zahra, F. (2023, April 27). *Nosocomial infections*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK559312/

42. Singh, S., Datta, S., Narayanan, K. B., & Rajnish, K. N. (2021). Bacterial exo-polysaccharides in biofilms: role in antimicrobial resistance and treatments. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 140. https://doi.org/10.1186/s43141-021-00242-y

43. Vareschi, S., Jaut, V., Vijay, S., Allen, R. J., & Schreiber, F. (2025). Antimicrobial efflux and biofilms: an interplay leading to emergent resistance evolution. *Trends in Microbiology*. https://doi.org/10.1016/j.tim.2025.04.012

44. Von Wintersdorff, C. J. H., Penders, J., Van Niekerk, J. M., Mills, N. D., Majumder, S., Van Alphen, L. B., Savelkoul, P. H. M., & Wolffs, P. F. G. (2016). Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.00173

45. Zhao, A., Sun, J., & Liu, Y. (2023). Understanding bacterial biofilms: From definition to treatment strategies. *Frontiers in Cellular and Infection Microbiology*, *13*. https://doi.org/10.3389/fcimb.2023.1137947

46. Zhao, W., You, J., Yin, S., Yang, H., He, S., Feng, L., Li, J., Zhao, Q., & Wei, L. (2022). Extracellular polymeric substances antibiotics interaction in activated sludge: A review. *Environmental Science and Ecotechnology*, *13*, 100212. https://doi.org/10.1016/j.ese.2022.100212

47. Zierke, L., Mourad, R., Kohler, T. P., Müsken, M., & Hammerschmidt, S. (2025). Influence of the polysaccharide capsule on virulence and fitness of Klebsiella pneumoniae. *Frontiers in Microbiology*, *16*. https://doi.org/10.3389/fmicb.2025.1450984