GVU-J.SHT *GVU Journal of Science, Health and Technology*

*GVU-J. SHT* 2025,**10**; **1**, 77–81, doi:10.5281/zenodo.15356929

# Bacterial Biofilms and Antimicrobial Resistance

**Abisoye, V. O.**[1*]

*Nigerian Institute for Oceanography and Marine Research, P. M. B. 12729, Victoria Island, Lagos, Nigeria*

***Received:*** *30 January 2025 []* ***Published:*** *13 April 2025*

Complex communities of microorganisms embedded in a self-generated extracellular matrix, known as biofilms, exhibit a universal tendency to adhere to surfaces. Globally, over 89% of microorganisms are organised in biofilms, prevalent in ecosystems such as surface water, lakes, and terrestrial environments. These structures demonstrate high resistance to antimicrobial agents, posing significant challenges in both environmental and clinical settings. This article examines the mechanisms by which biofilms confer antimicrobial resistance (AMR), focusing on biofilm production by Bacillus species on selected plants, the implications for public health, and potential strategies to address this issue.

**Keywords:** antimicrobial resistance, bacterial adhesion, matrix quorum sensing, microbial community, public health

## Introduction

Antimicrobial resistance (AMR) is recognised as an immediate and imperative global health challenge of the 21st century. AMR has been identified by the World Health Organisation (WHO) as a notable threat to public health, potentially compromising the effectiveness of antibiotics, which are pivotal in treating infections, surgical procedures, and managing chronic diseases. Among the various factors contributing to AMR, biofilms play a significant role (Mishra *et al.*, 2020).

Biofilms are complex microbiomes that attach to surfaces and are encased in a self-produced extracellular polymeric substance (EPS) matrix (Uruen *et al.*, 2021). This matrix provides structural stability and offers protection to the microorganisms within it. Biofilms can form on various surfaces, including natural environments, medical devices, and industrial equipment (Tajbakhsh *et al.*, 2016). Their inherent ability to adhere to surfaces and resist antimicrobial agents makes them particularly troublesome in clinical and environmental settings (Sharma *et al.*, 2019).

Biofilms are associated with persistent infections in clinical settings, such as those involving indwelling medical devices (e.g., heart valves and catheters), chronic wounds, and respiratory infections that damage the lungs and digestive system, as shown in Table 1 by the corresponding pathogen. These conditions are notoriously difficult to treat due to the enhanced resistance of biofilm-associated bacteria to antibiotics and the host immune response (Maale *et al.*, 2020). The presence of biofilms can lead to chronic infections, increased morbidity, prolonged hospitalisation, and higher healthcare costs.

The mechanisms by which biofilms contribute to antimicrobial resistance are complex and multifaceted. The EPS matrix acts as a physical barrier, restricting the penetration of antibiotics. Within the biofilm, bacteria can undergo phenotypic changes that enhance their resistance, such as reduced metabolic activity and increased expression of efflux pumps (Sharma *et al.*, 2023). Additionally, the proximity of bacteria within biofilms facilitates horizontal gene transfer, promoting the spread of resistance genes (Eberly *et al.*, 2018). The presence of persister cells, which are dormant and highly tolerant to antibiotics, further complicates treatment efforts. Recent research has highlighted biofilm formation on plants, particularly with the increasing use of pesticides, as shown in Table 2. For example, *Dickeya dadantii*, a gram-negative bacterium, causes soft rot diseases in a wide range of plant species.

**Table 1** Diversity of biofilm-producing bacteria on medical devices

| S/No | Pathogen | Medical Device |
|---|---|---|
| 1 | *Candida albicans* | Flexible endoscope |
| 2 | *Candida parapsilosis* | Intravascular devices |
| 3 | *Staphylococcus aureus* | Central nervous catheter |
| 4 | *Staphylococcus epidermidis* | Ureteroscope |
| 5 | *Pseudomonas aeruginosa* | Bronchoscope |

## Formation and Structure of Biofilms

Biofilms start to form when free-floating microorganisms adhere to a surface. These microorganisms produce extracellular substances known as the matrix that holds the biofilm together (Sharma *et al.*, 2023). This matrix not only guides the bacteria but also ensures communication and genetic transfer among them, as shown in Fig. 1 (Zhang *et al.*, 2020).

### *Steps of Biofilm Formation*

**Initial Attachment:** Van der Waals weak forces adhere the microorganisms to surfaces through pili and fimbriae (Awoke *et*

**Table 2** Biofilm bacteria on selected plants and their mechanisms of action

| S/No | Forming Bacteria | Host | Mechanism of Action |
|---|---|---|---|
| 1 | *Bacillus atrophaeus* | Biofilms on tomato | Biofilms produce surfactin and fengycin that are antimicrobial |
| 2 | *Bacillus subtilis* | Biofilms on wheat seeds | Preventing fungal microbial growth |
| 3 | *Paenibacillus polymyxa* | *Arabidopsis thaliana* | Biofilms provide protection to plants |
| 4 | *Bacillus amyloliquefaciens* SQR9 | Maize roots | Promote plant growth (PGP) activity |

*al.*, 2019).

**Irreversible Attachment:** An irreversible interaction occurs as microorganisms produce EPS, embedding themselves into a matrix (Dumaru *et al.*, 2019).

**Maturation:** The biofilm matures into a complex structure, generating a diverse environment accompanied by nutrient gradients and micro-colonies (Diriba *et al.*, 2020).

**Detachment/Dispersion:** Cells may leave the biofilm to revert to a planktonic state, propagating new biofilm emergence at a different location (Berne *et al.*, 2018).

## Mechanisms of Antimicrobial Resistance in Biofilms

Biofilms exhibit sophisticated mechanisms that contribute to a high rate of antimicrobial resistance. Here is a more detailed look at these mechanisms:

*Physical Barrier*

The extracellular polymeric substances (EPS) matrix, a distinctive feature of biofilms,(slot2) acts as a formidable barrier to the passage of antibiotics. This matrix, composed of proteins, lipids, extracellular DNA, and polysaccharides, limits the penetration of antimicrobial agents, effectively reducing their concentration before they reach the deeper layers of the biofilm where the majority of bacterial cells reside (Olaimat *et al.*, 2024).

*Altered Microenvironment*

Within a biofilm, the microenvironment is highly heterogeneous. Gradients of nutrients, pH, and oxygen levels create zones where bacteria can enter a slow-growing or dormant state. These physiological changes significantly reduce the efficacy of antibiotics, many of which target actively growing cells. For example, oxygen-depleted zones can shield anaerobic bacteria from antibiotics that require oxygen to be effective (Pai *et al.*, 2023).

*Genetic Exchange*

Biofilms facilitate close proximity among bacterial cells, enhancing horizontal gene transfer. This occurs through mechanisms such as transduction, transformation, and conjugation. The dense and stable environment of a biofilm supports the exchange of plasmids and other genetic elements that confer antibiotic resistance (Singh *et al.*, 2021).

*Phenotypic Changes*

Bacteria within biofilms can undergo significant phenotypic changes that increase their resistance to antibiotics. These changes include:

**Reduced Metabolic Activity:** In the deeper layers of the biofilm, bacteria often exhibit reduced activity, making them less susceptible to antibiotics that inhibit metabolic processes.

**Efflux Pumps:** Increased expression of efflux pumps can expel antibiotics from bacterial cells, lowering the intracellular concentration and efficacy of antibiotics (Yokoi *et al.*, 2024).

**Stress Responses:** Biofilm-associated bacteria can activate stress response pathways that enhance their survival under unfavourable conditions, including the presence of antibiotics (Adetunji *et al.*, 2021).

*Persister Cells*

Biofilms often contain a subpopulation of cells known as persisters. These dormant cells are highly tolerant to antimicrobial agents. Persisters are not genetically resistant but can survive antibiotic treatment and repopulate the biofilm once treatment ceases. This phenomenon contributes to the recurrent nature of biofilm-linked infections (Rather *et al.*, 2021).

*Quorum Sensing*

Quorum sensing is a cellular communication process that synchronises bacterial behaviour based on population density. It controls the expression of genes involved in antibiotic resistance and biofilm formation. Disrupting quorum sensing pathways is being explored as an alternative to combat biofilm-associated resistance (Sionov *et al.*, 2022).

## Clinical Implications of Biofilm-Associated Antimicrobial Resistance

Biofilm-related infections are challenging to eradicate. Infections involving indwelling medical devices, such as cardiac pacemakers, prosthetic joints, and catheters, often involve biofilms (Vandyck *et al.*,2021). Biofilms' resistance to antibiotics necessitates higher doses or prolonged treatment courses, which can lead to systemic toxicity and the development of antimicrobial resistance (Grooters *et al.*, 2024). Persistent infections, such as urinary tract infections, endocarditis, and osteomyelitis, are frequently associated with biofilms, complicating their management (Zafer *et al.*, 2024).

## Strategies to Combat Biofilm-Associated Antimicrobial Resistance (AMR)

Given the formidable challenges posed by biofilm-associated infections, innovative and multifaceted approaches are required

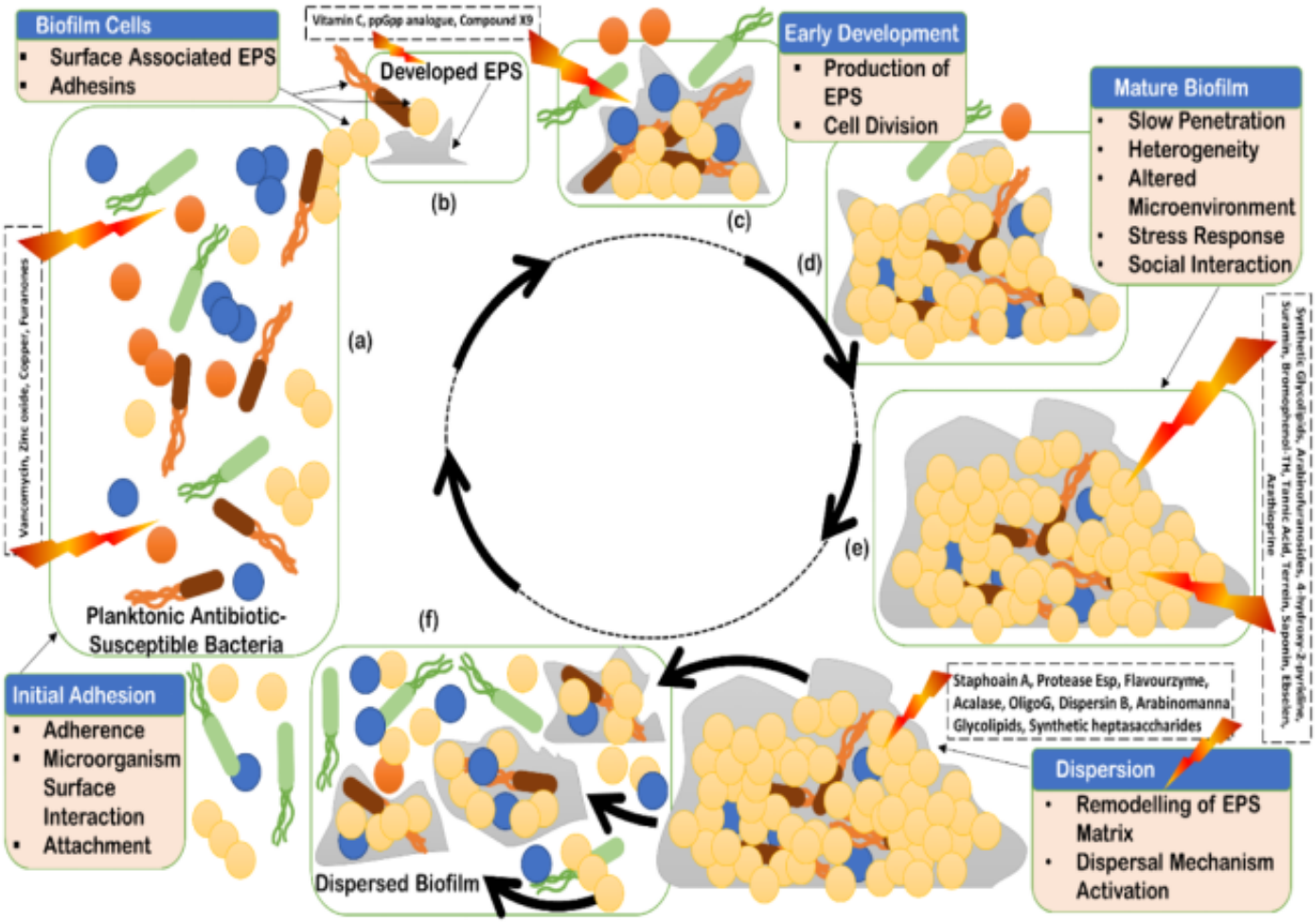


**Figure 1** Phases of biofilm life cycle: initial fixing (a); initial developed matrix (b); early development matrix production (c); further development of hydrogel to maturity (d); mature biofilm (e); dispersion of biofilm (Dutt *et al.*, 2022).

to effectively tackle their resilience and resistance. Conventional antibiotic therapies are often insufficient, necessitating novel strategies targeting various aspects of biofilm formation, persistence, and antimicrobial resistance. This section explores these advanced strategies, focusing on disrupting biofilm formation, enhancing antimicrobial efficacy, and developing alternative therapies that target biofilm-specific mechanisms.

*Extracellular Polymeric Substance (EPS) Disruption*

The EPS matrix, a dense protective barrier containing nucleic acids, polysaccharides, and proteins, is a major factor in resistance to antimicrobials. Disrupting this matrix can significantly enhance the penetration of antimicrobials, making the biofilm more vulnerable (Jiang *et al.*,2020).

**Enzymatic Degradation:** Enzymes such as DNase, proteases, and glycoside hydrolases can break down matrix components, weakening the biofilm structure. Studies have shown that combining these enzymes with antibiotics increases antibiotic efficacy by allowing deeper penetration into the biofilm (Jiang *et al.*,2020).

**Chelating Agents:** Compounds like ethylenediaminetetraacetic acid (EDTA) disrupt biofilms by binding to divalent metal ions, which are essential for maintaining biofilm structure. Removing these ions destabilises the matrix, enhancing antimicrobial activity (Sharma *et al.*,2023).

**Biosurfactants:** Rhamnolipids and sophorolipids demonstrate biofilm-disrupting capabilities by reducing surface tension and interfering with matrix production (Mishra *et al.*,2020). Their non-toxic and biodegradable properties make them favourable candidates for clinical applications.

*Inhibition of Quorum Sensing (QS)*

Quorum sensing (QS) is a communication process that enables bacteria to coordinate gene expression, including those responsible for biofilm production and virulence. Inhibiting quorum sensing can prevent biofilm formation or destabilise existing biofilms.

**Quorum Sensing Inhibitors (QSIs):** Several natural and synthetic compounds interfere with QS signalling. Plant-derived

QSIs, such as furanones and flavonoids, prevent biofilm formation and reduce antibiotic resistance (Sionov *et al.*,2022). These compounds disrupt QS signalling pathways, preventing bacteria from coordinating behaviours necessary for biofilm maturation.
**Autoinducer Analogs:** These molecules mimic chemical signals used in quorum sensing, binding to QS receptors and blocking genuine signals (Khatoon *et al.*,2018). By disrupting bacterial communication, these analogs inhibit biofilm formation and reduce pathogenicity.
**Enzymatic Degradation of Signalling Molecules:** Molecules like lactonases and acylases are being explored to inhibit biofilm formation by degrading signalling compounds used in bacterial communication (Sionov *et al.*,2022).

*Nanotechnology-Based Approaches*

Nanotechnology offers a novel approach to treat biofilm-associated AMR by utilising the properties of nanoparticles (NPs) to disrupt biofilms and enhance antimicrobial efficacy (Fulaz *et al.*,2019).

*Use of Bacteriophages*

Bacteriophages, viruses that specifically infect and kill bacteria, are gaining attention as therapeutic agents against biofilm-associated infections (Karygianni *et al.*,2020). Some phages produce enzymes, such as depolymerases, that degrade the matrix, making embedded bacteria susceptible to both phage attack and antibioticMotif: `attack`
Some phages produce enzymes, such as depolymerases, that degrade the matrix, making embedded bacteria susceptible to both phage attack and antibiotic treatment (Zafer *et al.*,2024). These enzymes target specific polysaccharides in the biofilm, breaking down the EPS matrix.
Combination phage therapy and antibiotics can produce synergistic effects, where phages disrupt the biofilm matrix and enhance antibiotic penetration (Akturk *et al.*,2023). This dual approach shows promise in reducing bacterial load in biofilm-associated infections, especially for antibiotic-resistant strains.
Advances in genetic engineering allow researchers to modify bacteriophages to enhance their effectiveness against biofilms (Flemming *et al.*,2022). For example, engineered phages can deliver enzymes that degrade biofilm components or target resistant bacterial strains more specifically, providing a tailored therapeutic option.

*Combination Therapies*

Combination therapies target different aspects of biofilm formation and resistance, enhancing overall treatment effectiveness.
Combining antibiotics with biofilm matrix-degrading enzymes, such as proteases or DNase, has shown improved outcomes in eradicating biofilm-associated infections (Wang *et al.*,2023). The enzymes degrade the matrix, allowing antibiotics to reach embedded bacteria.
Antimicrobial peptides (AMPs), naturally occurring proteins that disrupt bacterial cell membranes and inhibit biofilm formation, demonstrate synergistic effects when combined with traditional antibiotics, particularly against multidrug-resistant biofilm-forming pathogens (Tasneem *et al.*,2018).

## Conclusion

The complexity and resilience of biofilm-associated antimicrobial resistance present a formidable challenge in both clinical and environmental settings. However, the development of innovative strategies targeting the biofilm matrix, quorum sensing, persister cells, and resistant microbial populations offers hope for more effective treatment alternatives. Nanotechnology, phage therapy, and combination therapies represent promising avenues for overcoming the limitations of conventional antibiotics. As research continues to advance, a multidisciplinary approach that integrates these strategies could significantly mitigate the impact of biofilm-associated infections and antimicrobial resistance, addressing one of the most pressing global health challenges of the 21st century.

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