



Biofilm-Associated Antimicrobial Resistance and Tolerance in Pathogenic Bacteria: Molecular Mechanisms, Clinical Significance, and Emerging Therapeutic Strategies; A Review Article

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ABSTRACT

Biofilms are structured microbial communities in which bacterial cells adhere to biotic or abiotic surfaces and become embedded in an extracellular polymeric substance (EPS) matrix. Biofilm growth is closely associated with persistent and recurrent infections and with reduced antimicrobial susceptibility. This review synthesized current evidence on biofilm formation, biofilm-associated antimicrobial resistance and tolerance, clinically important biofilm-forming pathogens, and emerging antibiofilm strategies. The literature was considered through a narrative review approach, with emphasis on recent evidence concerning biofilm biology, resistance mechanisms, clinical significance, and therapeutic development. Particular attention was given to limited antimicrobial penetration, reduced metabolic activity, persister cells, efflux systems, altered gene expression, stress responses, quorum sensing, and horizontal gene transfer. Emerging approaches included bacteriophages and phage-derived enzymes, quorum-sensing inhibitors, matrix-degrading enzymes, antimicrobial peptides, nanoparticles, drug-delivery systems, combination therapy, and CRISPR-based approaches. The evidence indicated that biofilm-associated recalcitrance resulted from interacting physiological and genetic mechanisms rather than from a single resistance pathway. Although several antibiofilm interventions showed promising laboratory activity, translation to routine clinical practice remained limited by heterogeneity, delivery barriers, resistance development, safety considerations, and insufficient controlled clinical evidence.

CITATION

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INTRODUCTION

Antimicrobial resistance (AMR) is a major global public-health challenge. Multidrug resistant bacteria are becoming more common and are impacting the effectiveness of antimicrobial treatment, and making common infectious diseases more difficult to manage. The World Health Organization (WHO) 2024 Bacterial Priority Pathogens List defines 24 bacterial pathogens and

pathogen groups within 15 families as priorities for research, development and public-health interventions. Of significance are carbapenem-resistant *Acinetobacter baumannii*, resistant *Enterobacterales*, rifampicin-resistant *Mycobacterium tuberculosis*, carbapenem-resistant *P. aeruginosa*, methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *E. faecium* and resistant

Salmonella, Shigella and Neisseria gonorrhoeae (Liu et al., 2024).

One factor that is often overlooked, and is significant in contributing to antimicrobial treatment failure is the ability of the bacteria to form biofilms. Biofilms consist of populations of microorganisms that interact in a coordinated fashion, exchanged information, metabolic diversity, extracellular matrix and surface adhesion. Switching from planktonic growth to a biofilm lifestyle leads to significant differences in bacterial physiology, which enhances their persistence and resistance to antimicrobials (Choi et al., 2023; Shree et al., 2023; Zafer et al., 2024).

Recent studies have shown that antimicrobial resistance associated with biofilm is multifactorial. The extracellular matrix can block antimicrobial penetration, and slow growing or non-metabolizing cells can withstand antibiotic exposure. Furthermore, biofilms act as vehicles for gene transfer between cells, and conditions for resistance selection and persistence (Li et al., 2024; Sinha et al., 2024; Zafer et al., 2024).

Biofilm formation and antibiotic resistance are therefore not solely related to the acquisition of antibiotic resistance genes by bacteria. Instead, biofilms are considered to be a complex ecological system in which genetic resistance, phenotypic tolerance, physiological adaptation and intercellular communication converge (World Health Organization, 2024; World Health Organization, 2023; Li et al., 2024).

This review aims to synthesize current knowledge on the mechanisms of bacterial biofilm formation and biofilm-associated antimicrobial resistance and tolerance, examine the clinical significance of major biofilm-forming pathogens, and evaluate emerging strategies for the prevention and treatment of biofilm-associated infections. Antimicrobial resistance refers to a heritable decrease in antimicrobial susceptibility, whereas antimicrobial tolerance describes the ability of bacterial cells to survive antimicrobial exposure without necessarily showing an increase in the minimum inhibitory concentration. Persistence represents a transient phenotypic state in which a subpopulation survives antimicrobial exposure and can resume growth after the antimicrobial pressure is removed. Biofilm-associated recalcitrance is a broader phenotype produced by the combined effects of resistance, tolerance, persistence, physiological heterogeneity, matrix-associated protection, altered gene expression, and intercellular interactions.

Review Methodology

This article was prepared as a narrative review. The literature-search approach focused on recent and relevant evidence addressing bacterial biofilm formation, antimicrobial resistance and tolerance, clinically important biofilm-forming pathogens, and antibiofilm

interventions. PubMed, Scopus, Web of Science, and Google Scholar were considered as the principal literature sources. Search terms included combinations of “biofilm,” “biofilm formation,” “antimicrobial resistance,” “antibiotic tolerance,” “persister cells,” “quorum sensing,” “biofilm matrix,” “bacteriophage,” “antimicrobial peptide,” “nanoparticle,” “drug delivery,” and “CRISPR,” using Boolean operators such as AND and OR. Priority was given to peer-reviewed publications published from 2020 through 2025, while earlier seminal studies were retained when required to explain established mechanisms. Studies addressing bacterial biofilm biology, resistance or tolerance mechanisms, clinical relevance, prevention, or therapeutic strategies were considered relevant. Publications unrelated to bacterial biofilms, non-peer-reviewed material, and duplicate records were excluded. Titles and abstracts were screened for relevance, followed by full-text assessment when necessary. Because this was a narrative review rather than a systematic review, no formal meta-analysis or quantitative risk-of-bias synthesis was performed. The final evidence synthesis was organized thematically around biofilm development, matrix biology, quorum sensing, antimicrobial resistance and tolerance, clinically important pathogens, and emerging prevention and treatment strategies.

Biofilm Definition and General Characteristics

A biofilm can be described as an organized and structured community of microorganisms that are attached to a surface and enclosed in an extracellular matrix that is secreted by the microorganisms. Polysaccharides, proteins, extracellular DNA (eDNA), lipids and other extracellular materials are typically present in the matrix (Shree et al., 2023; Zafer et al., 2024). The bacteria found in biofilms are very different from the planktonic bacteria. They have changed gene expression, metabolic activity, stress tolerance and ability to communicate with other cells (Shree et al., 2023; Zafer et al., 2024).

The extracellular matrix gives the tissue its structural strength and makes a special environment. It can sequester nutrients, enzymes, signal molecules and extracellular DNA, and prevent immune and antimicrobial components from entering the host (Zafer et al., 2024).

Biofilms can form on:

- i. epithelial surfaces;
- ii. wounds;
- iii. gums, mouth and teeth;
- iv. urinary catheters;
- v. vascular catheters;
- vi. prosthetic joints;
- vii. cardiac devices;
- viii. endotracheal tubes;
- ix. medical implants; and
- x. Industrial and environmental surfaces.

Infections associated with biofilms are problematic because traditional susceptibility testing typically is based on planktonic bacterial cells and may not accurately measure the susceptibility of bacteria within a mature biofilm (Li et al., 2024; Shree et al., 2023).

The stages of Biofilm Formation

The development of a biofilm is generally believed to be a multistage process: reversible attachment, irreversible attachment, microcolony formation, maturation and dispersion (Shree et al., 2023; Zafer et al., 2024).

Initial Attachment

The first step is contact between planktonic bacterial cells and a surface. Surface characteristics, hydrophobicity, electrostatic interactions, environmental factors, and bacterial adhesions affect bacterial adhesion (Shree et al., 2023; Zafer et al., 2024). Flagella, pili, fimbriae, surface proteins, and other adhesions contribute to the initial attachment of bacterial cells (Shree et al., 2023).

Irreversible Attachment

After initial attachment, bacteria form extra-adhesive compounds and extracellular polymeric substances, which further fix their adhesion to the surface. The physical removal of bacteria cells at this stage is harder (Zafer et al., 2024).

Microcolony Formation

Bacteria attach and start to multiply and grow as microcolonies. The production of EPS increases, creating a structure and allowing the build-up of more bacterial cells (Zafer et al., 2024).

Biofilm Maturation

The biofilm forms a complex three-dimensional structure during maturation. Channels can develop within the matrix and help to distribute nutrients and remove wastes. Gradients of oxygen and nutrients occur throughout the biofilm, resulting in different bacterial cell populations (Shree et al., 2023; Zafer et al., 2024).

Dispersion

In the final stages, bacterial cells or cell clusters leave the mature biofilm, and can colonize new places. Dispersion helps spread infection and can start biofilms at sites far from the original infection in the body or environment. (Shree et al., 2023; Zafer et al., 2024)

The entire lifecycle of the biofilm can thus be described as: Attachment → Irreversible adhesion → Microcolony formation → Maturation → Dispersion

Recent mechanistic studies of antimicrobial resistance in biofilm are in line with this lifecycle (Choi et al., 2023; Li et al., 2024).

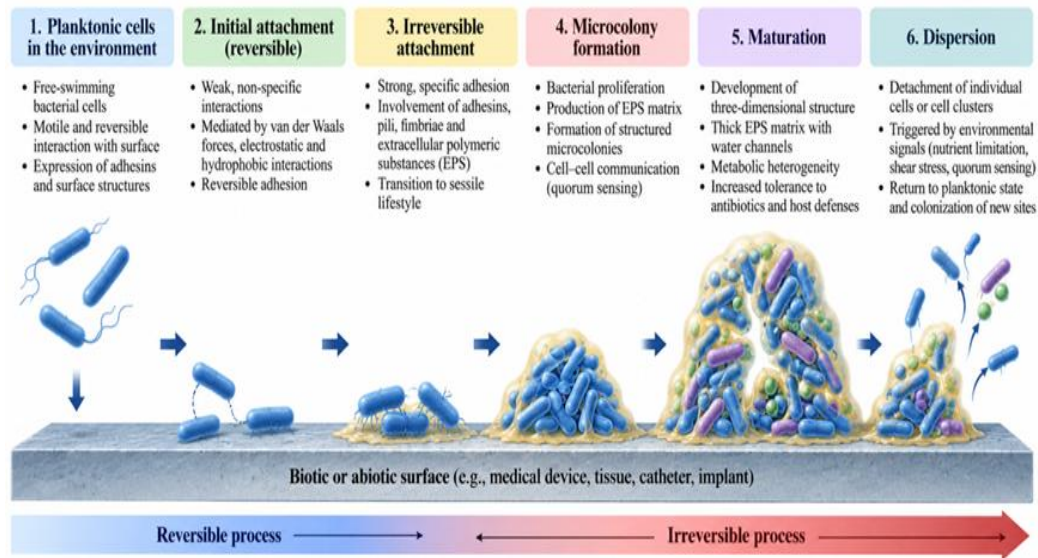


Figure 1: Multistage Process of Bacterial Biofilm Formation (Choi et al., 2023; Li et al., 2024)

Extracellular Polymeric Substance Matrix

The EPS matrix is one of the key elements of bacterial biofilms. It is mainly composed of polysaccharides, proteins, extracellular DNA, lipids and other macromolecules (Karballei Mirzahosseini et al., 2020; Zafer et al., 2024).

The matrix has several useful properties:

- i. provides structural stability;

- ii. facilitates bacterial adhesion;
- iii. retains nutrients and signaling molecules;
- iv. resists environmental stresses on bacterial cells;
- v. protects bacterial cells against antimicrobial agents and host immune defenses;
- vi. communicates between bacterial cells; and
- vii. Facilitates horizontal transfer of genes.

The extracellular DNA is especially significant since it can aid in matrix stability and can serve as a source of genetic material for horizontal gene transfer (Karballei Mirzahosseini et al., 2020; Zafer et al., 2024).

The matrix should not be considered as a simply physical barrier, though. It is an active biochemical environment, which plays a role in bacterial physiology, signaling, adaptation and resistance.

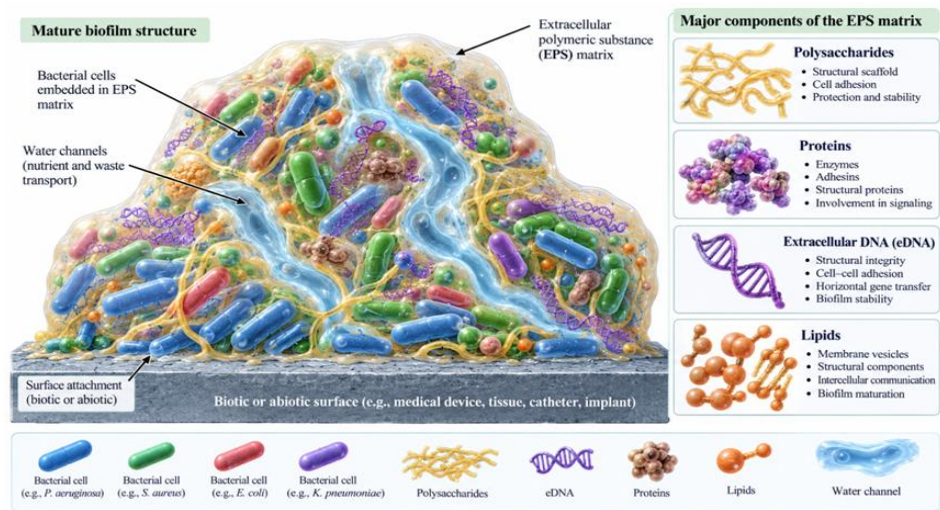


Figure 2: Structural Organization and Major Components of the Biofilm Extracellular Polymeric Substance Matrix (Karballei Mirzahosseini et al., 2020; Zafer et al., 2024)

Quorum Sensing and Biofilm Development

Enables bacteria to communicate with each other by producing, releasing, detecting and reacting to molecules called auto inducers, known as quorum sensing (QS) (World Health Organization, 2023). The higher the density of bacteria, the more molecules is produced and the higher the concentration can become until the molecules trigger coordinated changes in gene expression (World Health Organization, 2023).

QS Controls a number of processes related to biofilm biology, such as:

- i. adhesion;
- ii. EPS production;
- iii. virulence-factor expression;
- iv. motility;
- v. biofilm maturation;

- vi. dispersion; and
- vii. Stress adaptation.

Biofilm development and virulence in *Pseudomonas aeruginosa* is controlled by QS systems such as Las and Rhl and other regulatory networks. QS can also help enhance resistance to environmental stresses and antimicrobial agents. Recent research provides some evidence that combining QS inhibitors with conventional antibiotics may enhance susceptibility of biofilms (World Health Organization, 2023; Wang et al., 2022).

Thus, QS is an appealing antibiotic target: disrupting bacterial communication can help to inhibit the development of bacterial biofilms without necessarily killing the bacteria (World Health Organization, 2023; Karimaei et al., 2021).

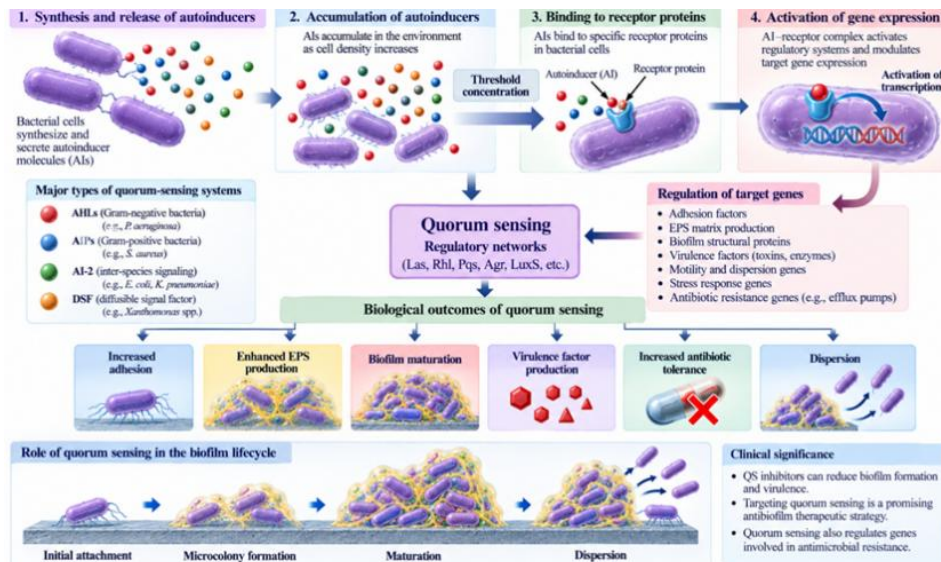


Figure 3: Quorum-Sensing Regulation of Biofilm Development and Bacterial Virulence (World Health Organization, 2023; Karimaei et al., 2021)

Mechanisms of Antibiotic Resistance and Tolerance in Biofilms

There are multiple overlapping mechanisms that contribute to the development of antimicrobial resistance associated with biofilms.

Limited Antibiotic Penetration

Some antimicrobial compounds may be less permeable to and diffuse through the extracellular matrix. Antibiotics can get physically trapped in, chemically altered in, and neutralized by the biofilm environment (Choi et al., 2023; Sinha et al., 2024). Although bioavailability is important for some elements of biofilm resistance, it is not all. Other physiological mechanisms make a significant contribution.

Reduced Metabolic Activity

Mature biofilms can have cells in the interior that are starved of nutrients and oxygen. This can markedly reduce their metabolic activity and growth rate (Li et al., 2024; Sinha et al., 2024). A number of antibiotics work best in growing cells. Thus, metabolically inactive cells can survive antimicrobial exposure, but do not necessarily have a heritable resistance mechanism.

Persister Cells

Persister cells are phenotypic variants that become metabolically dormant, or slow, and are highly resistant to antimicrobial therapy (World Health Organization, 2024; Singh et al., 2021). Once the concentration of the antibiotic drops, persister cells can restart growth and be a source of recurrent infection. This is an important difference: Resistance is usually defined as a decrease in susceptibility which is heritable while tolerance is a survival of bacteria in the presence of antimicrobial agents without necessarily resulting in an increase in the

minimum inhibitory concentration (MIC) (World Health Organization, 2024). Both of these can occur in a biofilm.

Efflux Pumps

Antimicrobial compounds are actively exported by efflux systems that decrease the amount of antibiotics in the bacteria. *P. aeruginosa*, *S. aureus* and other multidrug resistant (MDR) microorganisms have been targeted for their efflux pumps. An association between efflux systems and antibiotic resistance and biofilm development is observed in *S. aureus* (Iaconis et al., 2024; Singh et al., 2025).

Horizontal Gene Transfer

The high density of bacterial cells within a relatively stable matrix makes it ideal for genetic exchange as it is a favorable environment (Li et al., 2024; Zafer et al., 2024). Three major mechanisms of horizontal gene transfer are:

- i. conjugation;
- ii. transformation; and
- iii. Transduction.

Multi-drug resistance can thus be disseminated and/or acquired through the transfer of resistance determinants (da Silva et al., 2021; Li et al., 2024).

Altered Gene Expression

Bacteria undergo many changes in their transcriptional programs during biofilm growth. Some genes could be up- or down-regulated which do not affect the same part of the cell. Some genes may be up or down regulated, but do not affect the same part of the cell (Choi et al., 2023; Singh et al., 2025). Biofilm-associated bacteria encounter oxidative stress, nutrient limitation, pH variation, host immune factors, and antimicrobial exposure, and stress-response systems contribute to bacterial survival. Oxidative stress,

nutrient limitation, pH variations, host immune factors and antimicrobial exposure are common conditions that a biofilm-associated bacterium encounters. Stress response systems can promote the survival of bacteria (Li et al., 2024; Singh et al., 2025).

Recent reviews have focused on the synergistic combination of matrix production, slow growth, and modification of antibiotic targets, persister cells and resistance-gene exchange as the basis of the marked antimicrobial recalcitrance of biofilms (Choi et al., 2023; Li et al., 2024; Sinha et al., 2024).

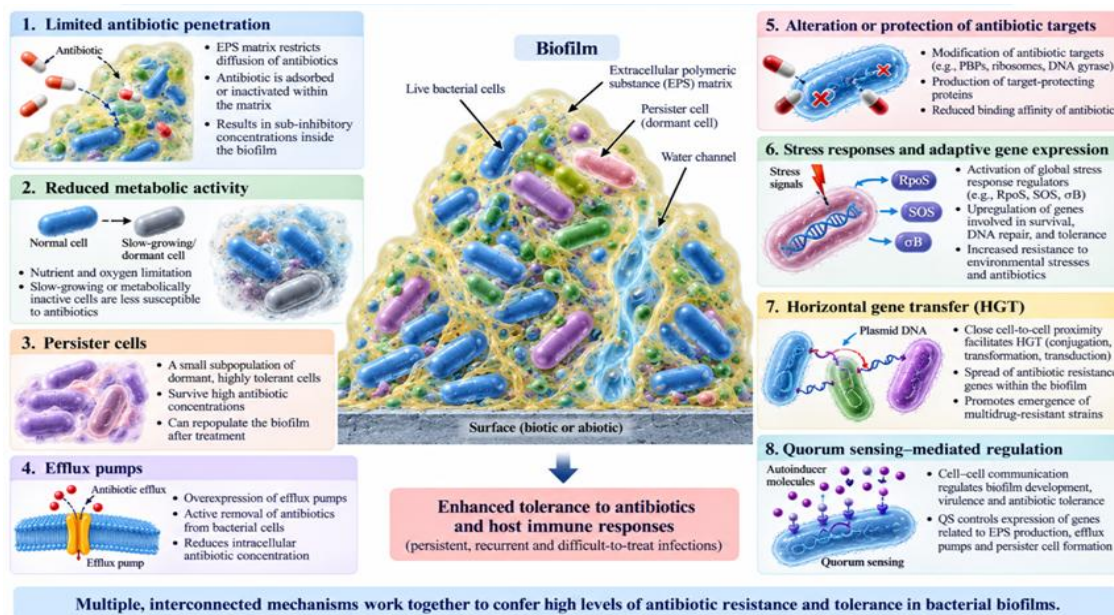


Figure 4: Molecular Mechanisms Underlying Antibiotic Resistance and Tolerance in Bacterial Biofilms (Choi et al., 2023; Li et al., 2024; Sinha et al., 2024)

Major Pathogenic Biofilm-Forming Bacteria

Staphylococcus aureus

S. aureus is a significant, Gram-positive, biofilm-forming pathogen on medical devices and tissues. MRSA poses a significant clinical threat as resistance to β -lactam antibiotics is associated with high β -lactam persistence as a biofilm component (Iaconis et al., 2024; Kılıç et al., 2023). Biofilm is associated with chronic wound infection, prosthetic device infection, osteomyelitis and infections associated with the use of a catheter (Kılıç et al., 2023; Zhao et al., 2020).

Pseudomonas aeruginosa

P. aeruginosa is an opportunistic, Gram-negative bacterium and one of the most significant biofilm forming bacteria. Chronic respiratory infections and infections of burns and medical devices are particularly important (Singh et al., 2025). The biofilm phenotype is controlled by the QS, c-di-GMP signaling, EPS production, motility and several stress-response pathways (World Health Organization, 2023; Singh et al., 2025).

Klebsiella pneumoniae

K. pneumoniae is a significant multidrug-resistant pathogen in the context of urinary tract infection (UTI),

pneumonia, bloodstream infection, liver abscess (Bhuiyan et al., 2025). Its persistence and resistance is aided by biofilm formation. The genetic control of biofilm formation and the link to antimicrobial resistance have been studied in recent years (Bhuiyan et al., 2025; da Silva et al., 2021).

Acinetobacter baumannii

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is classified by WHO as a critical-priority pathogen. It can persist on abiotic surfaces and form biofilms, contributing to healthcare-associated transmission and persistent infections (Abdelhamid et al., 2023; Liu et al., 2024).

Enterococcus faecium

VRE is a WHO High Priority Pathogen (HPP) in 2024. It has a tendency to survive in the healthcare environment and develop resistance determinants, which makes it a significant problem (Flemming et al., 2023; Liu et al., 2024).

Escherichia coli

Pathogenic *E. coli* can form biofilms in the urinary tract and urinary catheters. Recurrent UTI and failure to eradicate the infection with antimicrobial therapy is partly attributed to the formation of biofilm (Sharma et al., 2023).

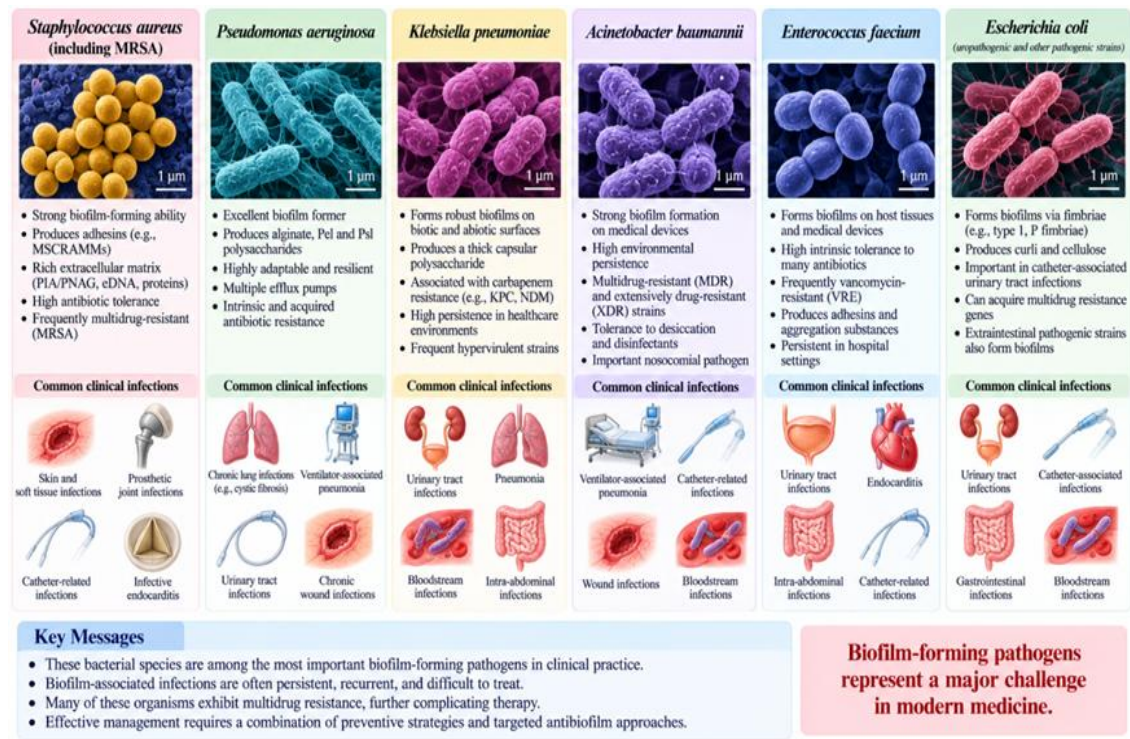


Figure 5: Major Pathogenic Bacteria Associated with Clinically Relevant Biofilm Infections (Sharma et al., 2023)

Clinical Significance of Biofilm-Associated Infections

There are some significant associations of biofilms with clinical conditions.

Table 1: Clinically Important Biofilm-Associated Infections and Their Major Bacterial Pathogens (World Health Organization, 2024; Weigelt et al., 2021; Zafer et al., 2024; Zhao et al., 2020)

Infection/condition	Important biofilm-forming organisms
Catheter-associated UTI	<i>E. coli</i> , <i>Klebsiella</i> spp., <i>P. aeruginosa</i> , <i>Enterococcus</i> spp.
Chronic wounds	<i>S. aureus</i> , <i>P. aeruginosa</i>
Cystic fibrosis lung infection	<i>P. aeruginosa</i>
Prosthetic joint infection	<i>S. aureus</i> , <i>S. epidermidis</i>
Central-line infection	<i>S. aureus</i> , <i>Enterococcus</i> spp., <i>Gram-negative bacilli</i>
Dental plaque	<i>Streptococcus</i> spp., <i>Actinomyces</i> spp.
Chronic otitis	Multiple bacterial species
Device-associated infections	ESKAPE pathogens

These organisms are also clinically relevant organisms, as highlighted by the WHO list of priority pathogens, where resistant *P. aeruginosa*, *S. aureus*, *E. faecium*, *A. baumannii* and resistant Enterobacterales are shown as a major global threat (Flemming et al., 2023; Liu et al., 2024).

Why Conventional Antibiotic Therapy May Fail

Conventional antibiotic treatment is commonly based on the susceptibility of planktonic bacteria. Bacteria in mature biofilms, however, can be quite different (Choi et al., 2023; Li et al., 2024; Sinha et al., 2024).

Failure in treatment can occur due to:

- i. inadequate antibiotic penetration;
- ii. low consumption of oxygen;

- iii. persister cells;
- iv. altered gene expression;
- v. increased efflux activity;
- vi. protection by the EPS matrix;
- vii. polymicrobial interactions;
- viii. horizontal gene transfer; and
- ix. Not enough drug level at the biofilm location.

Therefore it is important to note that just giving antibiotics more often is not adequate and can lead to more toxicity and selection pressure (Sinha et al., 2024; Srinivasan et al., 2021).

One of the key contemporary objectives is to find therapies which specifically target the structure and physiology of the biofilm (Kılıç et al., 2023; Weigelt et al., 2021).

Emerging Antibiofilm Strategies

Quorum-Sensing Inhibitors

QS Inhibitors disrupt bacterial communication and can inhibit biofilm formation, virulence and maturation (World Health Organization, 2023; Karimaei et al., 2021). A combination regimen of QS inhibitors and antibiotics has shown to be a potential approach as the ability to communicate may make bacteria more susceptible to antibiotic therapy.

Enzymatic Degradation of the Biofilm Matrix

Various enzymes, including DNases, dispersion B, proteases, and polysaccharide-degrading enzymes, have been studied for their effect on EPS components (Shenkutie et al., 2020; Szymczak et al., 2024). Degradation of the matrix can release antibiotic-sensitive bacterial cells from host immune mechanisms.

Bacteriophage Therapy

Bacteriophages are viruses which only infect bacteria. Lytic phages directly lyse bacterial cells and in a few instances produce enzymes that can break down parts of the matrix that forms biofilms (Mani et al., 2023; Visnapuu et al., 2022). However, host specificity and immune clearance, phage resistance, formulation, and regulatory issues are important concerns for phage therapy of multidrug-resistant organisms (Mani et al., 2023; Samrot et al., 2021). The promise of phages and phage derived enzymes as next generation antibiofilm therapies has been highlighted in recent reviews. (Mani et al., 2023; Pai et al., 2023)

Nanotechnology

Nanoparticles are able to potentially facilitate the delivery of antimicrobials and penetrate the biofilm structure (Belay et al., 2024; Jeong et al., 2024). Various antibiofilm

methods have been explored such as metal nanoparticles, polymeric nanoparticles, lipid-based systems, and stimuli-responsive nanomaterials (Belay et al., 2024; Jeong et al., 2024; Liu et al., 2022). Some of the major benefits of the more sophisticated drug-delivery systems are the potential for raising the concentration of antimicrobials at the site of action, while decreasing systemic exposure.

Antimicrobial Peptides

Antimicrobial peptides may be able to interact with bacterial membranes and may be active against planktonic and biofilm-associated cells (Szymczak et al., 2024). Some benefits they may offer are that they can have several modes of action and they are not reliant on the targets of classic antibiotics.

Combination Therapy

Combination approaches may involve:

- Antibiotic + phage
- Antibiotic + QS inhibitor
- Antibiotic + matrix-degrading enzyme
- Antibiotic + nanoparticle
- Phage + nanoparticle

This is to attack multiple parts of the biofilm at once (Kılıç et al., 2023; Weigelt et al., 2021).

CRISPR-Based Approaches

The CRISPR-associated systems are studied as precision antimicrobial agents that can target genes in the bacterial genome or resistance genes (Chang et al., 2022; Fernández-Billón et al., 2023; Flemming et al., 2023; Uruén et al., 2021; Wang et al., 2022). These approaches remain under development and offer potentially precise means of eradicating resistant bacterial populations.

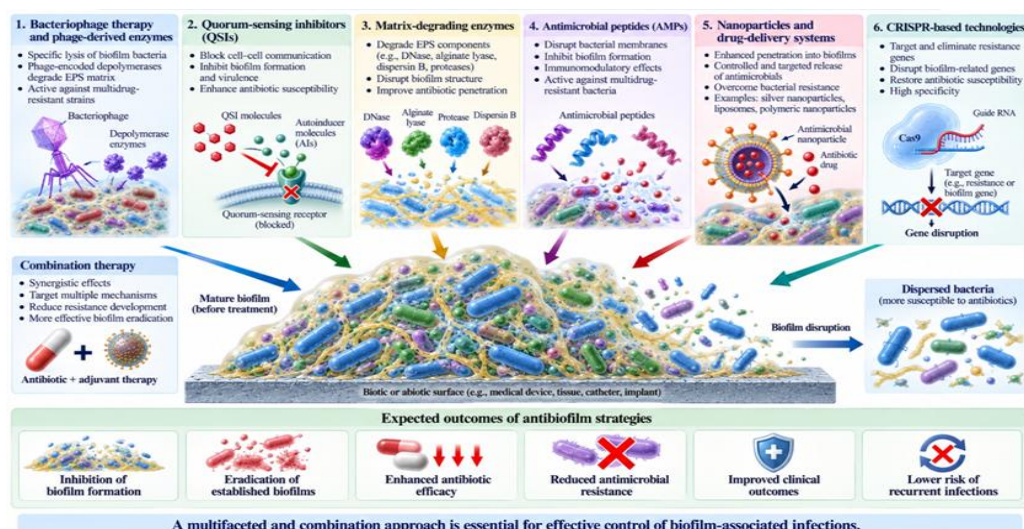


Figure 6: Emerging Antibiofilm Strategies for the Control of Persistent and Multidrug-Resistant Infections (Chang et al., 2022; Uruén et al., 2021)

Drug Delivery Strategies

Antibiofilm therapy has been a challenge, one of its major drawbacks, is the lack of delivery of therapeutic agents to the bacteria within the biofilm matrix (Belay et al., 2024; Li et al., 2020).

Modern approaches to delivery are:

- i. nanoparticles;
- ii. liposomes;
- iii. polymeric carriers;
- iv. hydrogels;

- v. surface coatings;
- vi. stimuli-responsive systems; and
- vii. Localized drug-delivery platforms.

These can help to improve the penetration into the biofilm, slow down degradation of therapeutic compounds, and facilitate accumulation of therapeutic compounds at the site of infection. Recent reviews have pinpointed drug delivery as one of the critical issues in the clinical translation of antibiofilm compounds (Belay et al., 2024; Li et al., 2020).

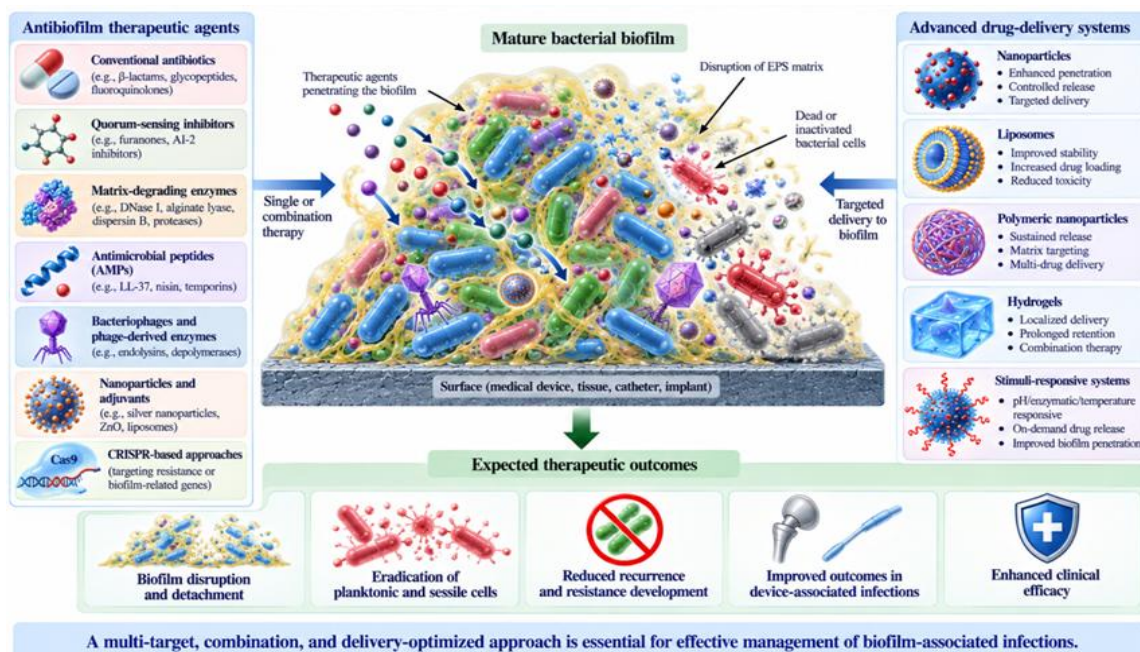


Figure 7: Integrated Biofilm-Targeted Therapeutic and Drug-Delivery Framework (Belay et al., 2024; Li et al., 2020)

Biofilm Control and Prevention

Prevention will also be important since mature biofilms are significantly more resistant to eradication (Grande et al., 2020; Kılıç et al., 2023).

Some crucial preventive strategies are:

- i. appropriate antimicrobial stewardship;
- ii. strict infection-control procedures;
- iii. appropriate catheter and device management;
- iv. surface modification of medical devices;
- v. Early detection of biofilm-associated infection;
- vi. appropriate wound management;
- vii. development of antibiofilm coatings;
- viii. Vaccination (when applicable); and
- ix. Minimizing unnecessary antimicrobial exposure.

WHO places a strong focus on infection prevention, better surveillance, vaccination, and the proper utilization of available treatment as important measures to control antimicrobial resistance, in addition to the development of new antibacterial drugs (Abebe et al., 2020; Liu et al., 2024).

Future Perspectives

Research should go beyond the traditional antibiotic susceptibility testing and more and more consider the biological nature of biofilm-associated bacteria (Choi et al., 2023; Shree et al., 2023).

There are important research priorities that include:

- i. standardized definitions of resistance; and
 - ii. development of molecular probes to detect the presence of biofilms; and
 - iii. Elucidation of the biology of persister cells;
 - iv. immunity to IGRs to develop effective QS inhibitors;
 - v. phage-antibiotic combinations;
 - vi. targeting delivery of antibiofilm agents;
 - vii. novel methods of removing or destroying biofilms from medical devices; and
 - viii. microbiome-based interventions;
 - ix. CRISPR-based antimicrobial technologies;
 - x. artificial-intelligence-assisted drug discovery; and
- The treatment of chronic biofilm-associated infections by addressing them in a personalized manner.

One of the biggest problems is to make promising findings from the laboratory into effective treatments in the clinic. There are numerous methods to fight biofilms, with many showing a highly potent effect in vitro but limited supporting data from controlled studies in humans (da Silva et al., 2021; Flemming et al., 2023; Samrot et al., 2021).

CONCLUSION

Biofilm-associated infections are characterized by antimicrobial recalcitrance arising from interacting mechanisms rather than from a single resistance pathway. Matrix-associated protection, physiological heterogeneity, reduced metabolic activity, persister cells, efflux systems, altered gene expression, stress responses, quorum sensing, and horizontal gene transfer collectively contribute to persistence and treatment failure. Clinically important organisms include *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, *E. faecium*, and pathogenic *E. coli*. Emerging interventions including bacteriophages, quorum-sensing inhibitors, matrix-degrading enzymes, antimicrobial peptides, nanoparticles, advanced drug-delivery systems, combination therapy, and CRISPR-based approaches provide complementary opportunities for biofilm control. However, promising laboratory activity has not consistently translated into routine clinical practice. Future priorities therefore include standardized biofilm definitions and susceptibility methods, improved biofilm-specific diagnostics, rigorous in vivo validation, well-designed clinical trials, optimized delivery systems, and integrated approaches that address both antimicrobial resistance and phenotypic tolerance.

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