Bacterial Biofilms and Antibiotic Resistance: Molecular Mechanisms and Emerging Therapeutic Strategies

Alyaa Mohammed Hasan Alghazi [1], Intisar Amer Hashim [1] , Mohammed N. F. Albadri [2], and Ahmed Alamiery [2,*]

[1]Dhi Qar Directorate of Education, 64001 Nasiriyah, Dhi Qar, Iraq.

[2]College of Pharmacy, Al-Ayen Iraqi University, AUIQ, 64001 Nasiriyah, Dhi Qar, Iraq.

[*]Corresponding Author

DOI: https://doi.org/10.51584/IJRIAS.2026.11100093

Received: 24 October 2026; Accepted: 29 October 2026; Published: 16 November 2026

ABSTRACT

Bacterial biofilms are spatially organized microbial communities whose extracellular matrix and physiological heterogeneity complicate the treatment of persistent infections. This review examines molecular mechanisms underlying biofilm-associated antibiotic resistance and tolerance and evaluates emerging therapeutic strategies in relation to their experimental evidence and translational limitations. Biofilm recalcitrance results from interacting processes rather than a universal protective mechanism. Extracellular polymeric substances influence antimicrobial distribution and host interactions, while oxygen and nutrient gradients generate physiologically distinct populations with variable antibiotic susceptibility. Regulatory signaling, efflux systems, stress responses, persister cells, and the selection or exchange of resistance determinants further contribute to survival. Importantly, heritable antibiotic resistance must be distinguished from phenotypic tolerance and persistence, which can reduce bacterial killing without necessarily increasing minimum inhibitory concentrations. Emerging approaches include matrix-disrupting enzymes, quorum-sensing and antivirulence inhibitors, antimicrobial peptides, nanocarrier-based delivery, bacteriophages, persister-directed interventions, and mechanism-guided combination treatments. Although these strategies demonstrate promise in laboratory and selected preclinical models, their clinical utility remains constrained by species-specific effects, biofilm heterogeneity, safety considerations, and limited comparative human evidence. Progress will require standardized viability and regrowth endpoints, infection-relevant models, and rigorous evaluation alongside established antimicrobial treatment and source control. A mechanism-informed, infection-specific approach may improve the development and assessment of therapies for biofilm-associated infections.

Keywords: Bacterial biofilms; antibiotic resistance; antibiotic tolerance; persister cells; extracellular polymeric substances; antibiofilm therapy; bacteriophages.

INTRODUCTION

Bacterial biofilms are structured microbial communities in which cells are embedded within a self-produced matrix of extracellular polymeric substances (EPS) and display physiological properties that differ from those of free-living, planktonic cells. Far from being restricted to clinical settings, biofilms are widespread in aquatic, terrestrial, and engineered ecosystems, where surface association provides a stable setting for nutrient exchange, cell–cell interactions, and adaptation to environmental stress. The biofilm matrix, consisting principally of polysaccharides, proteins, extracellular DNA, and lipids, supports adhesion and mechanical stability while creating a spatially organized microenvironment around resident cells [1]. In human infections, these same features may promote microbial survival, impair eradication, and contribute to disease persistence, establishing biofilm biology as a central concern in clinical microbiology [2].

Biofilm formation is commonly described as a dynamic sequence of initial attachment, establishment of more stable adhesion, microcolony formation, maturation, and dispersal. However, the duration and molecular

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue X October 2026

control of these stages differ among bacterial species and environmental conditions. Surface properties, nutrient availability, extracellular-matrix production, and regulatory pathways jointly determine the architecture of developing communities [3]. Quorum sensing is one such regulatory process: bacteria produce and detect extracellular signaling molecules that coordinate population-level gene expression and, in many species, influence virulence-associated behaviors and biofilm development [4]. Regulatory control is complemented by the matrix itself, which can alter interactions between biofilm-associated bacteria and host defenses; its molecular composition and protective effects vary across organisms and infection sites [5]. Quorum-sensing inhibitors have therefore attracted interest as potential anti-biofilm agents, although the dependence of biofilm formation on particular signaling systems is species- and context-specific [6].

The clinical importance of biofilms is particularly evident in chronic infections and infections involving implanted or indwelling medical devices. Biofilm-associated microorganisms can colonize catheters, prosthetic materials, and damaged or inflamed tissues, giving rise to infections that are difficult to resolve with antimicrobial treatment alone [2]. Representative pathogens include Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, and Escherichia coli, although biofilm-related pathogenicity depends on the organism, anatomical site, and host environment. Within these communities, sustained attachment, matrix-associated protection, and the survival of distinct physiological subpopulations may facilitate prolonged infection and recurrence following apparently successful treatment [7]. Such clinical behavior underscores the need to understand biofilms as heterogeneous microbial systems rather than simply dense collections of otherwise equivalent bacterial cells.

A defining therapeutic challenge is the markedly reduced susceptibility of many biofilm-associated populations to antibiotics. This phenotype is multifactorial and should not be equated automatically with heritable antibiotic resistance. The matrix can retard, bind, or inactivate particular antimicrobial compounds, but penetration limitations alone cannot explain the survival of all biofilms or all antibiotics [7]. Experimental work with P. aeruginosa demonstrated that the contribution of the MexAB–OprM efflux system to biofilm survival depended on the antibiotic and exposure conditions, illustrating the importance of mechanism-specific interpretation [8]. Efflux pumps can also affect microbial behaviors related to adhesion, signaling, and biofilm formation, thereby linking antimicrobial defense with community physiology [9]. Consequently, biofilm-associated treatment failure may reflect an interaction between conventional resistance determinants, altered antimicrobial exposure, and reversible physiological adaptations rather than any single universal mechanism.

Antibiotic tolerance and persistence are especially relevant to this distinction. Whereas resistance allows bacterial proliferation at antibiotic concentrations that inhibit susceptible counterparts, tolerance describes prolonged survival during exposure without necessarily increasing the minimum inhibitory concentration. Persister cells are phenotypic variants that can withstand otherwise lethal treatment and subsequently resume growth when the antimicrobial pressure is removed [10]. In vitro investigations of S. aureus biofilms have identified surviving subpopulations consistent with persistence after exposure to several antibiotics, supporting a role for physiological heterogeneity in biofilm recalcitrance [11]. Mathematical modeling has likewise shown how slow-growing regions of a biofilm may favor the accumulation and survival of persister-like cells, potentially permitting regrowth after treatment [12]. These findings are important because strategies directed solely toward classical resistance mechanisms may leave tolerant cells and biofilm architecture largely unaffected.

Together, biofilm architecture, regulatory networks, altered physiology, and antibiotic-response mechanisms create substantial challenges for infection control. Conventional antibacterial treatment may reduce the bacterial burden yet fail to eliminate established biofilm communities, motivating the development of agents that prevent attachment, destabilize the matrix, enhance antimicrobial delivery, or directly kill bacteria within mature biofilms [13]. Approaches that target multiple structural and functional features—including combination treatment and disruption of protected cellular niches—have been proposed as particularly relevant to this complex phenotype [14]. Anti-persister strategies are another active area of investigation, although experimental promise does not in itself establish clinical efficacy [15]. Recent studies of quorum-sensing regulation in P. aeruginosa [16] and of biofilm-modulating natural-product preparations in experimental

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue X October 2026

infection models [17] further illustrate the diversity of candidate targets, while emphasizing the distinction between laboratory findings and validated therapies.

As summarized in Figure 1, biofilm development gives rise to structural and physiological conditions that can diminish antimicrobial effectiveness and sustain infection, while also revealing targets for therapeutic intervention. This review therefore examines the molecular mechanisms underlying biofilm-associated antibiotic resistance and tolerance, focusing on EPS architecture, bacterial signaling, genetic and physiological determinants, efflux systems, and persister-cell formation. It then critically evaluates emerging therapeutic strategies, including novel antimicrobial agents, matrix-disrupting approaches, bacteriophage-based interventions, and combination regimens, with emphasis on their mechanistic rationale, current limitations, and translational prospects.

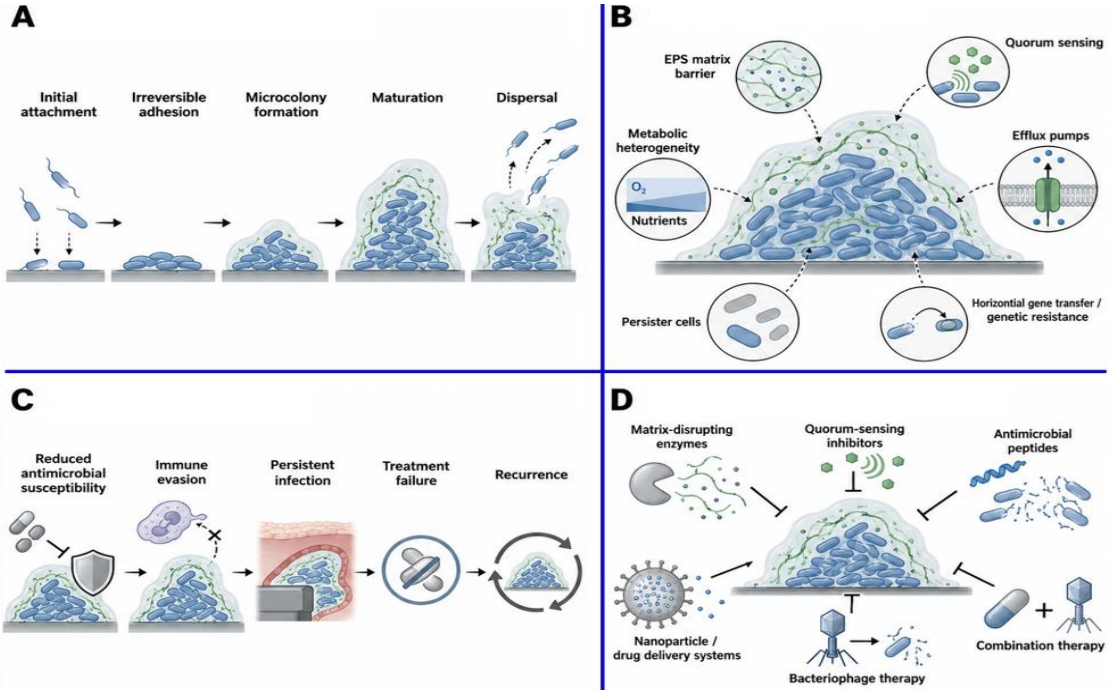


Figure 1. Overview of bacterial biofilm development, mechanisms of antibiotic resistance and tolerance, clinical consequences, and emerging therapeutic strategies. (A) Biofilm development proceeds through initial attachment, irreversible adhesion, microcolony formation, maturation, and dispersal. (B) Biofilm-associated antimicrobial recalcitrance involves multiple interacting mechanisms, including the extracellular polymeric substance (EPS) matrix, metabolic heterogeneity, quorum-sensing regulation, antibiotic efflux systems, persister-cell formation, and horizontal gene transfer. (C) These mechanisms contribute to reduced antimicrobial susceptibility, impaired immune clearance, persistent infections, treatment failure, and infection recurrence. (D) Emerging therapeutic approaches include matrix-disrupting enzymes, quorum-sensing inhibitors, antimicrobial peptides, nanoparticle-based drug delivery systems, bacteriophages, and combination therapies designed to target biofilm structure and bacterial survival.

Molecular Mechanisms of Biofilm-Associated Antibiotic Resistance

Biofilm Formation, Architecture, and Extracellular Polymeric Substances (EPS)

Biofilm development is a regulated and environmentally responsive transition from planktonic growth to a spatially structured community. Although attachment, microcolony development, maturation, and dispersal are frequently presented as sequential stages, this model does not imply that all species or infection settings follow an identical developmental program. Adhesins, appendages, surface properties, nutrient availability, and environmental stresses influence the initiation and persistence of bacterial aggregates. Once attached, bacterial cells produce and remodel extracellular polymeric substances (EPS) that stabilize surface association and provide a framework for community-level functions [1,2,3,18]. In addition to adherent surface-associated structures, clinically relevant bacterial aggregates can develop in host secretions and tissues, emphasizing that

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue X October 2026

biofilm organization cannot be defined solely by attachment to an inert material [2,14].

The EPS matrix contains variable combinations of exopolysaccharides, proteins, extracellular DNA (eDNA), and lipids. These constituents provide cohesion, retain water, promote interaction with host substrates, and can bind extracellular molecules. Their proportions and specific activities differ across organisms and growth conditions; consequently, no single matrix composition or permeability model adequately describes all pathogenic biofilms [1,5]. Matrix polymers can influence antibiotic distribution by electrostatic association, adsorption, and local chemical reactions. Nevertheless, physical obstruction should not be assumed to be the dominant mechanism for every drug–biofilm combination. Stewart emphasized the importance of diffusion geometry and reaction kinetics [19], whereas Walters and colleagues showed that ciprofloxacin and tobramycin entered an experimental Pseudomonas aeruginosa biofilm without producing extensive killing, implicating physiological rather than simply transport-based protection [20]. Biofilm architecture also establishes microenvironments with unequal access to oxygen, nutrients, metabolic substrates, and signaling molecules. In mature aggregates, diffusion limitations coupled with local consumption generate gradients that partition cells into physiologically distinct zones. Rapidly growing cells may coexist with slow-growing or relatively inactive cells within the same community, resulting in different susceptibility profiles even in the absence of stable genetic differences [21,20,22]. These spatial effects are particularly consequential for antimicrobials whose activity depends on active biosynthesis, membrane energetics, or cellular division. Figure 2 depicts the mechanistic relationship between matrix organization, resource gradients, and localized antibiotic responses.

Biofilm formation further requires coordination of cellular behaviors. Quorum-sensing circuits couple extracellular autoinducers to changes in transcription and, in selected species, regulate matrix production, virulence determinants, motility, and dispersal [4,6]. In P. aeruginosa, regulation of quorum sensing involves intersecting transcriptional networks: a recent study showed that altered PsrA expression affected LasR- and RhlR-associated signaling outputs, reinforcing the context-dependent nature of regulatory control [16]. The intracellular messenger cyclic di-GMP is another central component of bacterial transitions between motile and sessile states, although the relative contributions of signaling systems vary across organisms [3,21]. Regulatory inhibition must therefore account for pathway redundancy and for the possibility that disrupting a biofilm can release viable bacteria rather than eradicate them [6,13]. Matrix architecture and local signaling also contribute to immune evasion and interactions between coexisting organisms. The EPS layer can modify exposure to immune effectors, while localized nutrient competition, metabolic cooperation, and extracellular enzymes influence microbial fitness [1,5]. The clinical significance of these interactions extends to medical devices, chronic wound environments, and the airways of patients with chronic respiratory disease [2,23]. Collectively, structural organization is best understood not as a passive antibiotic shield but as an active ecological framework in which transport processes, cellular physiology, and gene regulation jointly determine antimicrobial outcomes [21,22].

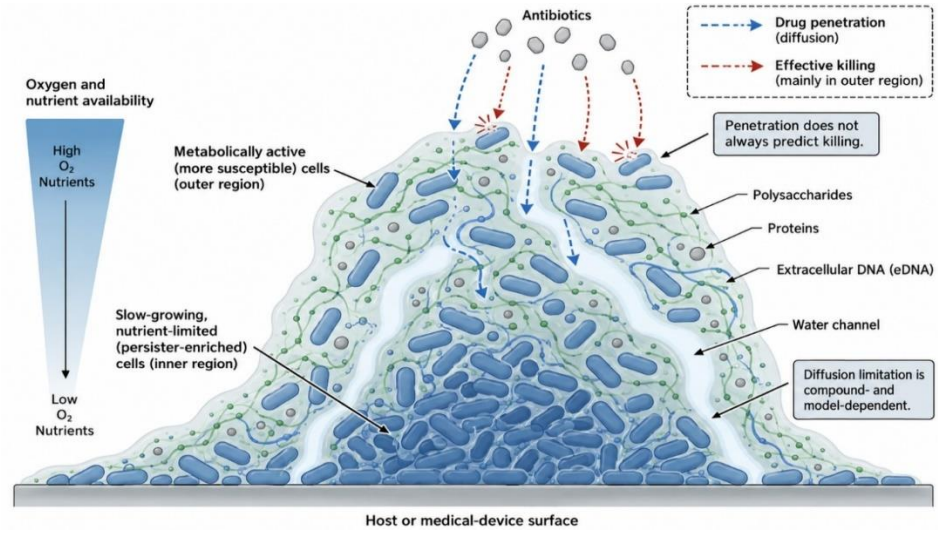


Figure 2. Schematic representation of bacterial biofilm architecture and spatial heterogeneity in antibiotic

exposure and susceptibility. The cross-sectional illustration depicts bacterial communities attached to a host tissue or medical-device surface and embedded within an extracellular polymeric substance (EPS) matrix composed of polysaccharides, proteins, and extracellular DNA (eDNA). Water channels and spatial gradients of oxygen and nutrient availability contribute to the formation of physiologically distinct bacterial subpopulations. Metabolically active cells are represented predominantly in the outer regions, whereas slower-growing and nutrient-limited cells occupy deeper regions of the biofilm. Differently patterned arrows distinguish antibiotic penetration from effective bacterial killing, emphasizing that antimicrobial entry does not necessarily result in bacterial eradication. The contribution of diffusion limitation to antibiotic tolerance varies according to the antimicrobial compound, matrix composition, bacterial species, and experimental conditions [1,5,19–22].

Genetic and Physiological Mechanisms of Antibiotic Resistance and Tolerance

A mechanistic discussion of biofilm-associated treatment failure requires a distinction between antibiotic resistance, tolerance, and persistence. Resistance describes a heritable ability to proliferate at concentrations that inhibit a susceptible population and is commonly assessed by growth-inhibition endpoints. Tolerance denotes prolonged survival during exposure to a bactericidal agent without necessarily increasing the minimum inhibitory concentration, whereas persistence usually refers to a subpopulation exhibiting especially prolonged survival in a killing assay. These phenomena may coexist but should not be used interchangeably [7,10,21,24]. Indeed, Spoering and Lewis found that the apparent superiority of biofilms over planktonic bacteria depended strongly on whether the comparator was actively growing or stationary-phase, illustrating how experimental design changes the interpretation of "biofilm resistance" [24].

Physiological heterogeneity is a major determinant of tolerance. Oxygen depletion, starvation, slow growth, and altered metabolic activity can decrease killing by antibiotics that rely on active cellular processes. Walters and colleagues correlated reduced killing in the deeper region of P. aeruginosa biofilms with oxygen limitation and lower metabolic activity [20]. However, metabolic inactivity is not universally protective: Pamp and colleagues observed colistin-tolerant metabolically active populations with phenotypes involving pmr and mexAB-oprM, and showed that antimicrobials acting on complementary subpopulations improved biofilm eradication in their model [25]. Such contrasting results demonstrate why drug-specific action and the spatial organization of susceptible and tolerant cells must be considered together.

Stress-response networks can reinforce these reversible states. In Escherichia coli biofilms, Bernier and colleagues linked starvation-associated ofloxacin tolerance to an SOS response and, under particular conditions, a contribution from stringent-response signaling [26]. Work on global regulators and nucleotide metabolism likewise implicated multiple regulatory functions in persister frequency, consistent with the view that persistence arises through heterogeneous and partially redundant routes rather than a universal single-gene program [27]. Persister survival has been investigated experimentally by population-killing kinetics and genetic perturbation; foundational studies linked toxin–antitoxin systems, including hipBA-associated regulation in E. coli, with changes in persister abundance [10,28]. However, these results do not establish that every toxin–antitoxin module is a necessary or sufficient determinant of persistence in all bacteria.

Studies of pathogenic organisms reveal additional species- and treatment-specific patterns. In S. aureus, persistent survivors and small-colony variants were observed in antibiotic-exposure experiments, although the two phenotypes showed different behaviors and should not be conflated [11]. In P. aeruginosa, Golmoradi Zadeh and colleagues measured survival and type II toxin–antitoxin gene expression after ciprofloxacin or colistin exposure, finding a greater persister fraction under stationary-phase conditions [29]. Patel and colleagues reported antibiotic-dependent patterns of persister survival and stress-associated gene expression in planktonic and biofilm populations [30]. Parallel studies of Brucella species [31] and the plant pathogen Xylella fastidiosa [32] support the broader relevance of toxin–antitoxin-associated stress responses, but extrapolation from those models to human biofilm infections requires caution. The potential for survivors to repopulate the biofilm after treatment is illustrated in the modeling study of Roberts and Stewart [12] and is integral to Figure 3.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue X October 2026

In addition to reversible tolerance, biofilms may favor the maintenance and evolution of heritable antibiotic resistance. Dense populations, extracellular DNA, population turnover, and antimicrobial gradients can create opportunities for selection and horizontal gene transfer, although transfer frequency and selective consequences depend on species and setting [22,33,34]. Babosan and colleagues showed that a qnrD-bearing plasmid in E. coli could affect biofilm formation and integron cassette rearrangements in a defined experimental context [33]. This provides an example of mechanistic coupling between mobile resistance elements and biofilm-associated traits, rather than evidence that all biofilms uniformly accelerate acquisition of resistance.

Antibiotic efflux systems constitute another important, but heterogeneous, component of this response. Efflux pumps can reduce intracellular antibiotic accumulation and can also serve functions in microbial physiology, signaling, and homeostasis [35]. Brooun and colleagues found a concentration- and antibiotic-dependent contribution of the MexAB-OprM system to fluoroquinolone survival in P. aeruginosa biofilms [8], while Alav and colleagues summarized links between efflux functions and biofilm formation across several pathogenic species [9]. Earlier investigations of fluoroquinolone efflux mechanisms likewise highlighted substantial organism-specific differences [8,9,17]. These findings argue against interpreting increased expression of an efflux gene, by itself, as proof of causality for biofilm drug tolerance. Table 1 consolidates the principal mechanisms, their evidence base, and the limitations that are relevant to designing mechanistically informed interventions.

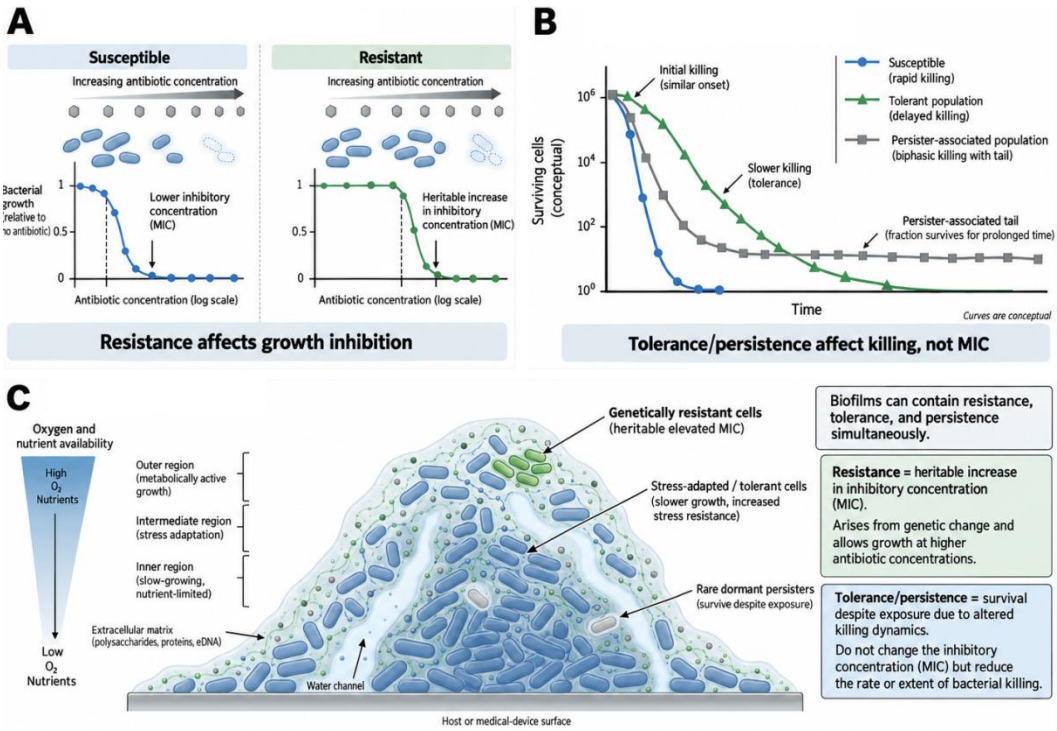


Figure 3. Conceptual model distinguishing antibiotic resistance, tolerance, and persistence within bacterial biofilms. (A) Schematic growth-inhibition profiles illustrate the difference between antibiotic-susceptible and genetically resistant bacterial populations. Heritable resistance is associated with an increased minimum inhibitory concentration (MIC), permitting bacterial growth at antibiotic concentrations that inhibit susceptible cells. (B) Idealized time–kill curves illustrate differences in bacterial survival during antibiotic exposure. Susceptible populations undergo relatively rapid killing, whereas tolerant populations exhibit slower killing without necessarily showing an increased MIC.

Persister-containing populations display biphasic killing kinetics characterized by an initial decline in viable cells followed by a slowly declining surviving fraction. (C) A biofilm cross-section illustrates the coexistence of genetically resistant cells, physiologically stress-adapted cells, and persister cells within a heterogeneous extracellular matrix. The figure highlights the distinction between heritable resistance and phenotypic survival mechanisms. All curves are conceptual representations rather than experimentally derived quantitative datasets [7,10,12,21,24,26,28].

Table 1. Mechanistic determinants of reduced antimicrobial susceptibility in bacterial biofilms, supporting evidence, and interpretive considerations.

Mechanism	Principal effect	Ref.	Interpretive limitation
EPS–drug interactions	Adsorption, sequestration, and context-dependent modification of local drug delivery	[1,5,21,19]	Penetration is drug-specific; the matrix does not universally exclude antibiotics.
Oxygen and nutrient gradients	Spatial differences in metabolic activity and antibiotic-mediated killing	[21,20,22]	Correlation with low metabolism does not establish the same mechanism for every agent.
Quorum sensing / regulatory signaling	Coordinates selected collective behaviors and stress responses	[3,4,16]	Effects on matrix production and antibiotic susceptibility differ by species.
Efflux transport	Can lower intracellular drug levels and influence biofilm-associated functions	[8,9,17,35]	Efflux contribution can be drug- and concentration-dependent.
Persisters / stress responses	Rare or enriched subpopulations survive bactericidal exposure and may regrow	[10,11,12,27,29,30,26,28]	Phenotypic survival does not necessarily signify heritable resistance.
Mobile resistance determinants	Genetic exchange and selection of resistant descendants	[22,33,34]	Demonstrated in particular systems; transfer rates cannot be generalized.
Spatially distinct adaptive responses	Different subpopulations tolerate different antibiotics	[38,25]	Combination efficacy depends on spatial, pharmacological, and biological context.

Emerging Therapeutic Strategies against Antibiotic-Resistant Biofilms

Novel Antimicrobial Agents and Biofilm-Disrupting Technologies

The multifactorial nature of biofilm-associated treatment failure favors strategies that target structural defenses and bacterial viability together. Conventional susceptibility testing of planktonic cells does not consistently capture matrix effects, physiological heterogeneity, or residual viable populations. Proposed antibiofilm therapies therefore aim either to prevent adhesion, impair maturation, disrupt established architecture, sensitize resident cells, or kill cells in otherwise protected microenvironments [3,13,14,36]. The distinction between preventing biofilm formation and eliminating an established, clinically relevant biofilm is particularly important: a compound that lowers crystal-violet staining or biomass in vitro may not achieve a comparable reduction in viable bacteria or infection burden [13,23].

Matrix-targeting treatments include enzymes that degrade major EPS constituents and approaches that weaken cohesion and improve access of antimicrobial agents. Disruption of extracellular DNA, polysaccharides, or proteinaceous matrix components has a strong mechanistic rationale because these materials determine biofilm integrity and interactions with antimicrobials and the host immune system [1,5,14]. However, enzymatic accessibility depends on matrix composition, species, and the infection environment. Dispersal should not be assumed to be synonymous with killing: surviving liberated cells could seed additional infection sites unless an effective companion antimicrobial or clearance mechanism is present [6,13]. This concern supports evaluating disruption treatments using both biomass and viable-cell endpoints.

Quorum-sensing interference offers an alternative means of altering virulence-associated coordination and, in certain organisms, biofilm maturation. Small molecules that suppress production, perception, or downstream

action of extracellular signals have demonstrated antibiofilm activity in experimental systems [6]. The regulatory complexity of Gram-negative quorum sensing, including intersecting signal–response networks, provides both multiple intervention points and multiple routes through which efficacy might vary [4,16]. For example, a 2025 study of a Phyllanthus emblica seed extract reported altered quorum-sensing-associated behavior and improved survival of an infected Caenorhabditis elegans model; nevertheless, its composite extract and model-system design do not establish clinical utility for a purified antibiofilm drug [17]. Similar caution applies to extrapolating pathogen-specific quorum-sensing experiments to mixed-species infections.

Antimicrobial peptides, polymer-based carriers, and nanoparticle platforms are being investigated to enhance local drug delivery, damage bacterial envelopes, and interfere with the matrix or biofilm-associated physiology [14,36,37]. Nanoparticles may permit payload protection, controlled release, or surface modification, but their activity depends on physicochemical properties, host-derived proteins, tissue penetration, and toxicity. For antimicrobial peptides, stability in biological fluids and potential host-cell toxicity remain important development challenges. Reviews of P. aeruginosa-directed strategies describe promising results for peptides, nanomaterials, photodynamic approaches, and quorum-sensing antagonists, while emphasizing that evidence remains predominantly experimental for many candidates [37]. Broad treatment proposals should therefore be separated from demonstrated clinical outcomes.

Photodynamic and other localized physical approaches provide an additional strategy for damaging bacteria through externally activated processes. These may be attractive for accessible surfaces or localized infections, but efficacy is constrained by light access, tissue penetration, biofilm depth, and compatibility with the surrounding host environment [14,37]. Across emerging platforms, reproducible antimicrobial delivery and activity within host-relevant matrices remain major bottlenecks. Figure 4 integrates structural and physiological targets with potential treatment modalities, while Table 2 evaluates the comparative maturity and limitations of the proposed interventions.

Biological and Combination Therapies for Biofilm Eradication

Bacteriophages and phage-derived enzymes are under investigation because they may selectively infect susceptible bacteria or degrade particular matrix components. Their potential value includes amplification at permissive infection sites and, for some phages or depolymerases, direct impairment of biofilm architecture [14,36]. Yet host-range restrictions, bacterial resistance to phages, variable activity in spatially heterogeneous biofilms, immune interactions, and manufacturing standardization complicate clinical translation. Phage-associated results must be interpreted according to whether the intervention used intact replicating phages, a purified enzyme, or a defined combination, because these modalities have different pharmacological and regulatory requirements [36,23].

Combination therapy is particularly attractive when interventions address different biofilm compartments or complementary physiological states. Pamp and colleagues showed in an in vitro P. aeruginosa system that combining colistin with antibiotics preferentially active against distinct bacterial subpopulations resulted in substantially greater eradication than single agents [25]. Similarly, Chua and colleagues described colistin-tolerant populations that employed motility and quorum-sensing-associated processes; a treatment combining colistin with erythromycin enhanced eradication in their experimental model [38]. These findings demonstrate the potential value of targeting adaptive transitions and spatially separate populations, but they do not justify assuming that the same combinations will be effective or safe across all pathogens and infection sites.

Targeting persisters provides a further rationale for combination regimens. Because persister-associated survival is typically phenotypic and treatment-dependent, approaches under investigation include metabolic reactivation, sensitization of stress-response pathways, and agents that retain killing activity against cells with low biosynthetic activity [10,15]. The complexity of these processes is evident from contrasting findings in E. coli, P. aeruginosa, and S. aureus, where persister formation depends on the experimental state, drug, and genetic background [11,27,29,30,26,28]. A clinically useful anti-persister therapy would need to reduce the surviving fraction without simply stimulating uncontrolled bacterial outgrowth or selecting resistant clones. Validation should include time–kill assays, post-treatment regrowth, testing in host-relevant systems, and comparison against standard care [15].

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue X October 2026

Broader combinatorial strategies pair antibiotic treatment with EPS-disrupting enzymes, local delivery vehicles, quorum-sensing inhibitors, phages, or physical interventions. Reviews of experimental antibiofilm therapies recognize that combined targeting may improve access and bacterial killing, but also underline the limitations of using laboratory biomass reduction as the primary measure of success [13,14,36]. Specifically, polymicrobial composition, immune factors, tissue architecture, and drug pharmacokinetics can change the outcomes obtained in simple static in vitro assays [23]. Infection-specific optimization will therefore be essential: a catheter-associated biofilm, chronic wound, and cystic-fibrosis airway aggregate differ substantially in accessibility, microbial ecology, and available adjunctive care [2,37,23].

Therapeutic comparisons also require methodological consistency. Useful endpoints include viable-cell reduction, reduction in regrowth after treatment, antibiofilm activity in mature versus developing communities, changes in resistance emergence, host-cell compatibility, and efficacy in validated infection models. Reporting only prevention of attachment or reduction of total biomass risks overstating clinical relevance [13,14,36]. Advances in imaging, spatially resolved analysis, and biofilm-specific susceptibility approaches may help identify which mechanistic subgroup is being affected and improve the selection of rational combinations [21,23]. Ultimately, therapies should be prioritized according to reproducible in vivo benefit and an acceptable safety profile rather than novelty of the delivery platform alone.

A central translational principle emerges from the evidence surveyed: biofilms are not single, homogeneous drug targets. Matrix-associated protection, metabolically inactive cells, actively adaptive populations, genetically resistant lineages, and dispersing bacteria may coexist within one infection. Successful treatment may therefore require coordinated intervention across several biological compartments, tailored to pathogen and infection site, and combined with source control when clinically indicated [2,14,21]. Figure 4 presents the proposed integration of target-directed approaches, and Table 2 provides a structured comparison of the main candidate modalities and the evidence limitations that should guide future preclinical and clinical evaluation.

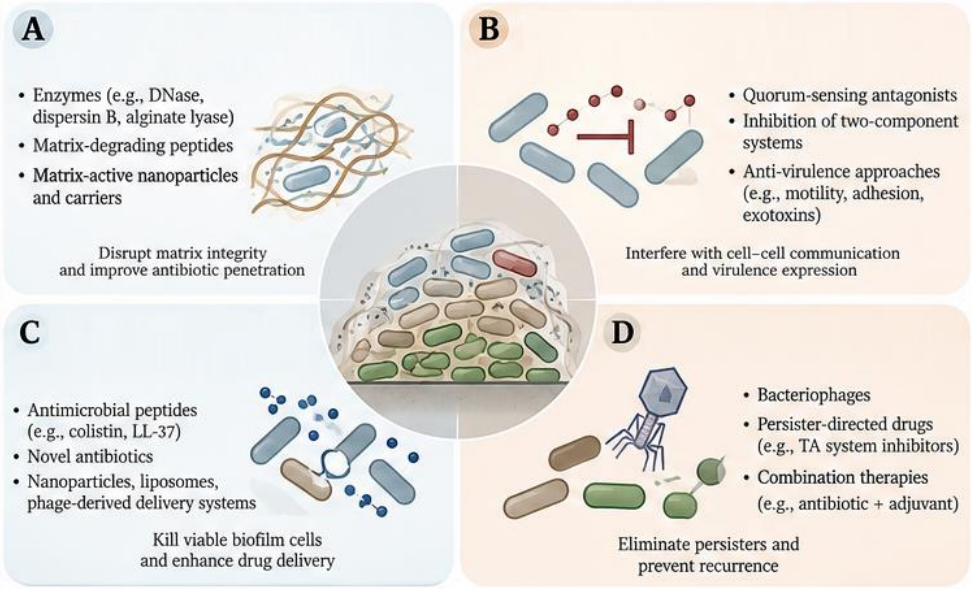


Figure 4. Mechanism-guided overview of emerging therapeutic strategies targeting bacterial biofilms. (A) Extracellular polymeric substance (EPS)-degrading enzymes and matrix-active delivery systems target biofilm structural integrity and may facilitate antimicrobial access to embedded bacterial cells. (B) Quorum-sensing antagonists and antivirulence agents interfere with bacterial communication pathways and selected regulatory processes involved in biofilm development and pathogenicity. (C) Antimicrobial peptides and targeted antibacterial delivery systems aim to enhance bacterial killing and improve drug distribution within biofilm communities. (D) Bacteriophages, persister-directed agents, and combination therapies target protected bacterial subpopulations and mechanisms associated with persistent or recurrent infections. The accompanying evidence band distinguishes established clinical infection-management components from experimental adjunctive interventions and preclinical therapeutic concepts. Most emerging antibiofilm approaches remain investigational, and their clinical effectiveness requires further validation in relevant infection models and

controlled clinical studies [6,13–15,17,23,25,36–38].

Table 2. Emerging antibiofilm approaches: biological targets, reported promise, and barriers to clinical translation.

Therapeutic approach	Mechanistic rationale / supporting sources	Key translational considerations
EPS-degrading enzymes	Weaken matrix components to improve structural disruption and, potentially, antibiotic access [1,5,14].	Matrix specificity; residual viable bacteria; dispersal and dissemination risk.
Quorum-sensing inhibitors	Alter selected population-coordination and virulence pathways [4,6,16,17].	Regulatory redundancy; species specificity; limited evidence in human infection.
Antimicrobial peptides	Target bacterial envelopes and potentially biofilm-resident cells [14,37].	Host toxicity, stability, local drug concentration, and delivery.
Nanoparticle or carrier systems	Improve local delivery or co-delivery of antibacterial and matrix-active components [14,36,37].	Biodistribution, biocompatibility, formulation reproducibility.
Photodynamic interventions	Localized energy-driven damage to microbial cells [37].	Light access, depth, selectivity, and tissue compatibility.
Phages and phage-derived enzymes	Lysis of susceptible bacteria or degradation of selected matrix components [14,36,23].	Host range, resistance to phages, standardization, host responses.
Persister-directed strategies	Reduce non-growing survivors or enhance killing after sensitization [10,15,27,29,30].	Risk of regrowth, model dependence, and demonstration of in vivo efficacy.
Combination regimens	Target distinct mechanisms and subpopulations concurrently [14,38,25].	Interaction may be context-dependent; dosing, toxicity, and clinical benefit require validation.

CONCLUSION

Biofilm-associated infections remain difficult to treat because bacterial survival within structured communities is governed by multiple, interacting mechanisms. The extracellular polymeric substance matrix provides mechanical organization and alters the local chemical environment, whereas nutrient and oxygen gradients generate populations with different growth rates and antibiotic responses. Quorum-sensing and other regulatory pathways, efflux systems, stress adaptations, and mobile genetic elements further shape biofilm behavior. These processes must be interpreted with a clear distinction between heritable antibiotic resistance and phenotypic tolerance or persistence. In particular, the survival of persister-enriched or otherwise stress-adapted subpopulations may permit regrowth after treatment even when the bacteria do not exhibit a sustained increase in minimum inhibitory concentration. The therapeutic implications of this heterogeneity extend beyond the search for stronger antibiotics. Enzymatic matrix disruption, quorum-sensing interference, antimicrobial peptides, improved drug-delivery platforms, bacteriophage-based approaches, and combinations targeting different physiological compartments offer complementary strategies. Nevertheless, reductions in biomass or improvements in killing in simplified experimental systems should not be equated with proven clinical eradication. Their effectiveness depends on the pathogen, biofilm architecture, infection site, drug exposure, and host environment, while dispersal of viable cells, toxicity, and resistance evolution remain important considerations. Future investigations should prioritize standardized and clinically meaningful endpoints, including viable-cell reduction, post-treatment regrowth, safety, and efficacy in mature biofilms and host-relevant infection models. Comparative preclinical studies and well-designed clinical trials are needed to establish which combinations provide benefits beyond existing antimicrobial therapy and appropriate source control. Overall, translating antibiofilm research into effective care will require an integrated understanding of

matrix biology, microbial physiology, genetic resistance, and the practical constraints of infection-specific treatment.

REFERENCES

1. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8:623–633. doi:10.1038/nrmicro2415.
2. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15(2):167–193. doi:10.1128/CMR.15.2.167-193.2002.
3. Rabin N, Zheng Y, Opoku-Temeng C, Du Y, Bonsu E, Sintim HO. Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Med Chem. 2015;7(4):493–512. doi:10.4155/fmc.15.6.
4. Papenfort K, Bassler BL. Quorum sensing signal–response systems in Gram-negative bacteria. Nat Rev Microbiol. 2016;14:576–588. doi:10.1038/nrmicro.2016.89.
5. Gunn JS, Bakaletz LO, Wozniak DJ. What's on the outside matters: the role of the extracellular polymeric substance of Gram-negative biofilms in evading host immunity and as a target for therapeutic intervention. J Biol Chem. 2016;291(24):12538–12546. doi:10.1074/jbc.R115.707547.
6. Brackman G, Coenye T. Quorum sensing inhibitors as anti-biofilm agents. Curr Pharm Des. 2015;21(1):5–11. doi:10.2174/1381612820666140905114627.
7. Mah TF. Biofilm-specific antibiotic resistance. Future Microbiol. 2012;7(9):1061–1072. doi:10.2217/fmb.12.76.
8. Brooun A, Liu S, Lewis K. A dose-response study of antibiotic resistance in Pseudomonas aeruginosa biofilms. Antimicrob Agents Chemother. 2000;44(3):640–646. doi:10.1128/AAC.44.3.640-646.2000.
9. Alav I, Sutton JM, Rahman KM. Role of bacterial efflux pumps in biofilm formation. J Antimicrob Chemother. 2018;73(8):2003–2020. doi:10.1093/jac/dky042.
10. Lewis K. Persister cells. Annu Rev Microbiol. 2010;64:357–372. doi:10.1146/annurev.micro.112408.134306.
11. Singh R, Ray P, Das A, Sharma M. Role of persisters and small-colony variants in antibiotic resistance of planktonic and biofilm-associated Staphylococcus aureus: an in vitro study. J Med Microbiol. 2009;58:1067–1073. doi:10.1099/jmm.0.009720-0.
12. Roberts ME, Stewart PS. Modelling protection from antimicrobial agents in biofilms through the formation of persister cells. Microbiology. 2005;151:75–80. doi:10.1099/mic.0.27385-0.
13. Verderosa AD, Totsika M, Fairfull-Smith KE. Bacterial biofilm eradication agents: a current review. Front Chem. 2019;7:824. doi:10.3389/fchem.2019.00824.
14. Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L. Targeting microbial biofilms: current and prospective therapeutic strategies. Nat Rev Microbiol. 2017;15:740–755. doi:10.1038/nrmicro.2017.99.
15. Defraine V, Fauvart M, Michiels J. Fighting bacterial persistence: current and emerging anti-persister strategies and therapeutics. Drug Resist Updat. 2018;38:12–26. doi:10.1016/j.drup.2018.03.002.
16. Kok LC, Tsai CC, Liao YH, Lo YL, Cheng NW, Lin CT, Chang HY. Roles of transcriptional factor PsrA in the regulation of quorum sensing in Pseudomonas aeruginosa PAO1. Front Microbiol. 2024;15:1424330. doi:10.3389/fmicb.2024.1424330.
17. Balkrishna A, Ngpoore NK, Bhatti S, Varshney Y, Kabdwal M, Nain P, et al. Linolenic acid-enriched supercritical carbon dioxide extract from seeds of Phyllanthus emblica L., a functional food with quorum quenching and anti-biofilm action, rescues Caenorhabditis elegans infection model from Pseudomonas aeruginosa. Appl Food Res. 2025;5:101244. doi:10.1016/j.afres.2025.101244.
18. Satpathy S, Sen SK, Pattanaik S, Raut S. Review on bacterial biofilm: an universal cause of contamination. Biocatal Agric Biotechnol. 2016. doi:10.1016/j.bcab.2016.05.002.
19. Stewart PS. Diffusion in biofilms. J Bacteriol. 2003;185:1485–1491. doi:10.1128/JB.185.5.1485-1491.2003.
20. Walters MC III, Roe F, Bugnicourt A, Franklin MJ, Stewart PS. Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of Pseudomonas aeruginosa biofilms to ciprofloxacin and tobramycin. Antimicrob Agents Chemother. 2003;47:317–323. doi:10.1128/AAC.47.1.317-323.2003.

21. Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev. 2017;41:276–301. doi:10.1093/femsre/fux010.
22. Uruén C, Chopo-Escuin G, Tommassen J, Mainar-Jaime RC, Arenas J. Biofilms as promoters of bacterial antibiotic resistance and tolerance. Antibiotics. 2021;10:3. doi:10.3390/antibiotics10010003.
23. Grari O, Ezrari S, El Yandouzi I, Benaissa E, Ben Lahlou Y, Lahmer M, et al. A comprehensive review on biofilm-associated infections: mechanisms, diagnostic challenges, and innovative therapeutic strategies. The Microbe. 2025;8:100436. doi:10.1016/j.microb.2025.100436.
24. Spoering AL, Lewis K. Biofilms and planktonic cells of Pseudomonas aeruginosa have similar resistance to killing by antimicrobials. J Bacteriol. 2001;183:6746–6751. doi:10.1128/JB.183.23.6746-6751.2001.
25. Pamp SJ, Gjermansen M, Johansen HK, Tolker-Nielsen T. Tolerance to the antimicrobial peptide colistin in Pseudomonas aeruginosa biofilms is linked to metabolically active cells, and depends on the pmr and mexAB-oprM genes. Mol Microbiol. 2008;68:223–240. doi:10.1111/j.1365-2958.2008.06152.x.
26. Bernier SP, Lebeaux D, DeFrancesco AS, Valomon A, Soubigou G, Coppée JY, et al. Starvation, together with the SOS response, mediates high biofilm-specific tolerance to the fluoroquinolone ofloxacin. PLoS Genet. 2013;9:e1003144. doi:10.1371/journal.pgen.1003144.
27. Hansen S, Lewis K, Vulić M. Role of global regulators and nucleotide metabolism in antibiotic tolerance in Escherichia coli. Antimicrob Agents Chemother. 2008;52:2718–2726. doi:10.1128/AAC.00144-08.
28. Keren I, Shah D, Spoering A, Kaldalu N, Lewis K. Specialized persister cells and the mechanism of multidrug tolerance in Escherichia coli. J Bacteriol. 2004;186:8172–8180. doi:10.1128/JB.186.24.8172-8180.2004.
29. Golmoradi Zadeh R, Sadeghi Kalani B, Mahdizade Ari M, Talebi M, Razavi S, Masjedian Jazi F. Isolation of persister cells within the biofilm and relative gene expression analysis of type II toxin/antitoxin system in Pseudomonas aeruginosa isolates in exponential and stationary phases. J Glob Antimicrob Resist. 2022;28:30–37. doi:10.1016/j.jgar.2021.11.009.
30. Patel H, Buchad H, Gajjar D. Pseudomonas aeruginosa persister cell formation upon antibiotic exposure in planktonic and biofilm state. Sci Rep. 2022;12:16151. doi:10.1038/s41598-022-20323-3.
31. Amraei F, Narimisa N, Sadeghi Kalani B, Lohrasbi V, Masjedian Jazi F. Persister cells formation and expression of type II toxin-antitoxin system genes in Brucella melitensis (16M) and Brucella abortus (B19). Iran J Pathol. 2020;15:127–133. doi:10.30699/ijp.2020.118902.2294.
32. Merfa MV, Niza B, Takita MA, De Souza AA. The MqsRA toxin-antitoxin system from Xylella fastidiosa plays a key role in bacterial fitness, pathogenicity, and persister cell formation. Front Microbiol. 2016;7:904. doi:10.3389/fmicb.2016.00904.
33. Babosan A, Gaschet M, Muggeo A, Jové T, Skurnik D, Ploy MC, et al. A qnrD-plasmid promotes biofilm formation and class 1 integron gene cassette rearrangements in Escherichia coli. Antibiotics. 2022;11:715. doi:10.3390/antibiotics11060715.
34. Schroeder M, Brooks BD, Brooks AE. The complex relationship between virulence and antibiotic resistance. Genes. 2017;8:39. doi:10.3390/genes8010039.
35. Martínez JL, Sánchez MB, Martínez-Solano L, Hernández A, Garmendia L, Fajardo A, Alvarez-Ortega C. Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. FEMS Microbiol Rev. 2009;33:430–449. doi:10.1111/j.1574-6976.2008.00157.x.
36. Grooters KE, Ku JC, Richter DM, Krinock MJ, Minor A, Li P, et al. Strategies for combating antibiotic resistance in bacterial biofilms. Front Cell Infect Microbiol. 2024;14:1352273. doi:10.3389/fcimb.2024.1352273.
37. Singh P, Arsi L. Pseudomonas aeruginosa biofilms: pathogenicity, clinical challenges and emerging therapeutic strategies. The Microbe. 2026;10:100645. doi:10.1016/j.microb.2025.100645.
38. Chua SL, Yam JKH, Hao P, Adav SS, Salido MM, Liu Y, et al. Selective labelling and eradication of antibiotic-tolerant bacterial populations in Pseudomonas aeruginosa biofilms. Nat Commun. 2016;7:10750. doi:10.1038/ncomms10750.