International Journal of Environment, Agriculture and Biotechnology
Vol-11, Issue-5; Sep-Oct, 2026
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Basics of Biofilm Architecture and Antimicrobial Resistance

Subrat Kumar Barik[1*], Priti Sundar Mohanty[1,2,3], Mugdha Sinha[1], Shailesh Rout[1]

[1]School of Biotechnology, Kalinga Institute of Industrial Technology (KIIT) Deemed to be University, Bhubaneswar 751024, India
[2]School of Chemical Engineering, Kalinga Institute of Industrial Technology (KIIT) Deemed to be University, Bhubaneswar 751024, India
[3]Centre for Soft Matter & Bio-Engineering (CSMBE), Kalinga Institute of Industrial Technology (KIIT) Deemed to be University, Bhubaneswar 751024, India
*Corresponding Author: bariksubratkumar19@gmail.com

Received: 03 Aug 2026; Received in revised form: 31 Aug 2026; Accepted: 04 Sep 2026; Available online: 19 Sep 2026

Abstract— *The most common type of microbial life in nature is found in bacterial biofilms, which are organized colonies attached to surfaces. They are thought to be the cause of up to 80% of human chronic illnesses and over two-thirds of microbiological infections. The global antimicrobial resistance (AMR) epidemic is intimately related to their clinical relevance; in 2021, bacterial AMR was linked to an estimated 4.71 million deaths worldwide. Unlike traditional genetic resistance, biofilm-associated resistance largely stems from the structural and chemical composition of biofilms. The extracellular polymeric substance (EPS) matrix, its three-dimensional organization, and the internal chemical heterogeneity are the main topics of this review, which summarizes the current knowledge on biofilm architecture. It investigates the mechanisms—diffusion limitation, metabolic heterogeneity, efflux pump activity, improved horizontal gene transfer, and quorum-sensing regulation—by which this architecture confers antimicrobial resistance and tolerance.. The clinical impact of biofilm-associated infections in medical devices, chronic wounds, and the airways of cystic fibrosis patients is discussed along with emerging therapeutic strategies targeting biofilms, including quorum-sensing inhibitors, matrix-degrading enzymes, nanotechnology-based delivery systems, and bacteriophage therapy. We conclude that effective management of infections associated with biofilms necessitates strategies that target multiple aspects of biofilm architecture, used in combination with, rather than as a replacement for, conventional antimicrobial therapy.*

Keywords— ***Biofilm, extracellular polymeric substance (EPS), antimicrobial resistance, quorum sensing, persister cells, antibiofilm therapy***

I. INTRODUCTION

Throughout the majority of the twentieth century, bacterial research predominantly focused on free-floating planktonic cells within laboratory cultures. However, it is now well recognized that this does not reflect the natural state of bacteria. Most bacteria exist as biofilms, which are surface-associated, multicellular communities where cells are irreversibly attached to a substratum and to each other, embedded within a self-produced extracellular matrix (1). Researchers and physicians' perspectives on recurrent and persistent infections have been profoundly impacted by this paradigm shift.

Biofilms are increasingly acknowledged as substantial contributors to the global antimicrobial resistance (AMR) crisis, a factor that has been historically underestimated. The magnitude of this crisis is significant. A 2024 study estimated that bacterial AMR was associated with 4.71 million deaths worldwide in 2021, with 1.14 million directly attributable to resistant infections (2). An earlier study attributed 1.27 million deaths in 2019 to antibiotic-resistant infections (3). The World Health Organization's 2024 Bacterial Priority Pathogens List highlights the organisms posing the most significant threat, with Gram-negative bacteria and rifampicin-resistant *Mycobacterium*

tuberculosis identified as critical priorities for research and development (4).

Biofilms are intricately involved in various aspects of clinical practice related to microbial infections. According to estimates by the United States National Institutes of Health, biofilms are implicated in approximately 65% of all microbial infections and up to 80% of chronic human infections (5,6,7). Bacteria residing in a biofilm can tolerate antibiotic concentrations that are significantly higher in some reported cases up to a thousand-fold higher than those required to eliminate genetically identical planktonic cells (8). Importantly, this increased tolerance is usually not due to the acquisition of new resistance genes by biofilm-embedded bacteria. Instead, it is the architecture of the biofilm including its matrix, internal chemistry, and the physiological states it induces in resident cells that actively confers protection. Antimicrobial tolerance is a reversible physiological state that allows cells to withstand drug exposure without changing their genetically determined susceptibility, whereas antimicrobial resistance is a persistent heritable feature encoded by mutation or an acquired resistance gene. (9). Biofilms facilitate this phenomenon. They establish conditions that favour short term tolerance, and, over time, enhance the probability of the emergence and dissemination of true genetic resistance among cells, and even across species (10).

This review initially explores biofilm architecture, focusing on their structural composition. We then delve into the specific mechanisms by which this architecture contributes to antimicrobial resistance and tolerance. Additionally, this review evaluates the clinical burden of biofilm-associated infections and examines novel strategies aimed at directly targeting biofilm architecture directly, rather than relying solely on conventional antibiotics.

II. BIOFILM FORMATION: FROM PLANKTONIC CELLS TO STRUCTURED COMMUNITIES

Biofilm formation is a highly regulated developmental process distinct from bacterial aggregation. Using *Pseudomonas aeruginosa*, the most extensively studied biofilm-forming pathogen, as a model organism, the process is typically divided into five stages (fig.1): reversible attachment, irreversible attachment, maturation I, maturation II, and dispersion (11).

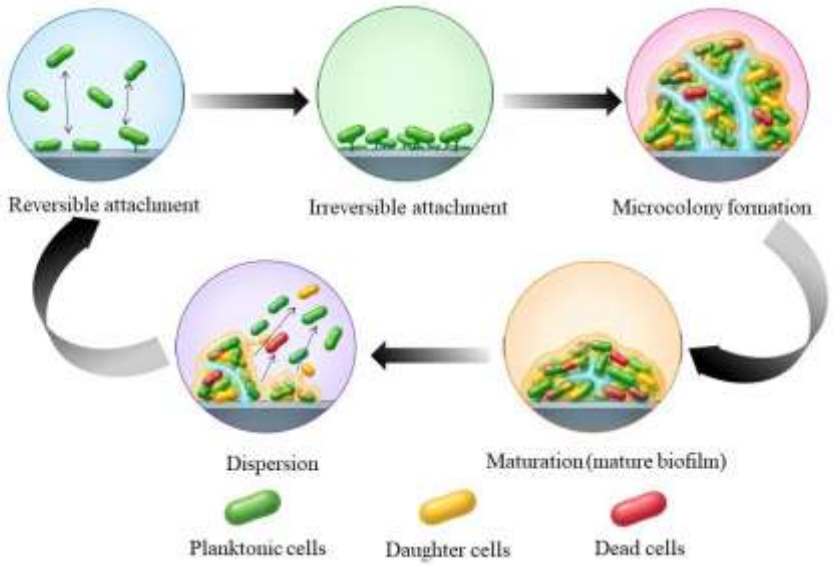

Fig. 1: The Stages of Biofilm Formation (Generated using AI)

Through flagella-mediated movement and general physicochemical interactions, planktonic cells first make weak contact with the surface during the reversible attachment phase. The cells can still separate at this point and return to their free-swimming state (11). The cells move toward irreversible attachment as contact becomes more stable. During this transition, flagellar gene expression is downregulated, type IV pili's surface engagement is increased, and extracellular polymeric substance (EPS) secretion is initiated, all of which help build the biofilm matrix (11). Cyclic di-GMP, an intracellular second messenger, is essential for controlling this transition. While higher amounts promote matrix formation and adherence to a sessile, biofilm-associated lifestyle, low intracellular cyclic di-GMP levels promote mobility and dispersal. Microcolonies are created when cells multiply and attract

more cells after irreversible attachment. During maturation stages I and II (11), these microcolonies develop into distinctive three-dimensional, frequently mushroom-shaped structures of mature biofilms. A subset of cells in the final dispersion stage are prompted by intracellular and environmental cues to change back to a motile planktonic phenotype, which allows them to actively leave the biofilm and colonize new locations. Clinically, dispersion has two sides: while it is essential for the biofilm life cycle and environmental dissemination, it also makes it easier for a persistent, localized biofilm infection to develop into bacteraemia or metastatic infection at distant locations.

III. *ARCHITECTURE OF THE BIOFILM*: THE EXTRACELLULAR MATRIX AND BEYOND

3.1 Composition of the EPS matrix

The matrix of a biofilm is a self-produced, hydrated network of extracellular polymeric substances (EPS) that sets it apart from a bacterial collection. The resident cells, which usually make up the bulk of the biofilm volume, are embedded and connected by this matrix. Flemming and Wingender describe the matrix as aiding adherence to surfaces, offering mechanical stability, and functioning as a "external digestive system." This tactic enables the community to digest biopolymers that would not otherwise be accessible by maintaining extracellular enzymes near to the cells that produce them (12).

The chemical heterogeneity of the matrix is mostly composed of lipids, proteins, extracellular DNA (eDNA), and polysaccharides. Different species, strains, and even growth conditions have very different relative amounts of these components. The most common structural component is often polysaccharides. diverse exopolysaccharides serve diverse purposes in different organisms. For instance, certain adhesins help with initial surface attachment, whereas others provide bulk structural resilience and cohesiveness to the mature biofilm (13). Notable examples include Pel and Psl in Pseudomonas aeruginosa, alginate, bacterial cellulose, and poly-N-acetylglucosamine. Extracellular DNA (eDNA), hitherto believed to be unintentional debris from lysed cells, is now recognized as an essential structural component. It can form stable filamentous networks that interact with other matrix constituents and directly contribute to the overall architecture and mechanical integrity of a strikingly diverse variety of bacterial and fungal biofilms (14). Owing to its structural significance, enzymatic degradation of eDNA is an active target for anti-biofilm therapy, as discussed in Section-7.

3.2 Three-dimensional architecture and spatial heterogeneity

Beyond its chemical composition, the matrix forms a distinct three-dimensional architecture of mushroom- or pillar-shaped microcolonies interspersed with interstitial voids and channels that facilitate the movement of water, nutrients, and metabolic waste. These channel networks are not merely open pathways (Fig. 2). A recent study on *Escherichia coli* biofilm transport channels shows that some are enclosed within thin layers of actively growing cells that maintain distinct internal chemical gradients along the channel, rather than being directly exposed to the external environment (15).

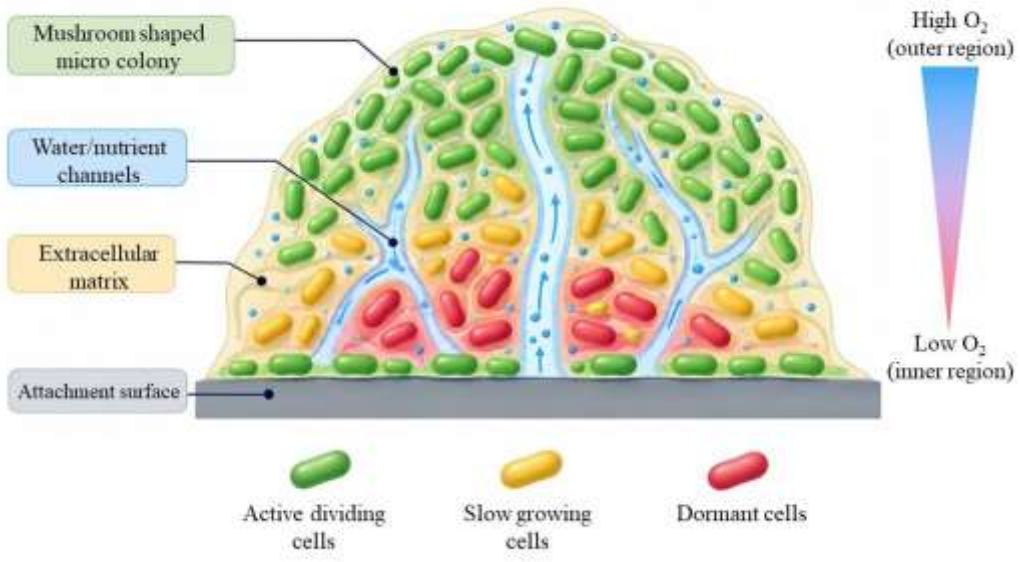

Fig.2: EPS Matrix Architecture & Internal Gradients (Generated using AI)

This architecture introduces significant physicochemical heterogeneity within a single biofilm. Oxygen, nutrients, and antimicrobial agents diffuse inward from the biofilm surface and are gradually consumed or bound as they penetrate deeper, generating steep gradients between an oxygen-abundant, nutrient-rich periphery and an often hypoxic or anoxic, nutrient-depleted core (15). Recent multiscale modelling, which considers the bacterial capsule as a distinct low-diffusivity layer within the bulk matrix, estimates that this fine-scale architecture can decrease local oxygen transfer by approximately 70% compared to a matrix without this structural feature, functioning as a "resistance-in-series" barrier to diffusion (16).

The physiological consequences of this heterogeneity are profound. Various metabolic states are adopted by cells in various micro-regions within the same biofilm because they are exposed to significantly varying local environments. For example, cells in the centre may develop slowly or remain dormant, whereas those near the oxygenated perimeter may divide quickly. This internally produced metabolic variability is one of the key ways that biofilm architecture provides antibiotic tolerance, as covered in Section-5.

IV. TECHNIQUES FOR STUDYING BIOFILM ARCHITECTURE

Understanding biofilm architecture depends heavily on the imaging tools available to study it. Confocal laser scanning microscopy (CLSM) is widely regarded as the single most important technique in the field as it is non-destructive, allowing biofilms to be examined *in situ*, in their native hydrated state, and imaged repeatedly over time (17). Combined with fluorescent- or lectin-conjugated stains specific to individual matrix components, CLSM allows researchers to map the spatial distribution of specific matrix constituents in relation to the cells and to each other (17).

Quantitative analysis of CLSM image stacks, including biomass, thickness, surface roughness, and porosity, is typically performed with dedicated image-analysis software. However, methodological standardization remains a persistent challenge in the field, as automated thresholding procedures may introduce systematic bias unless rigorously validated against the underlying image data. To construct a more comprehensive understanding of biofilm architecture, complementary techniques such as scanning electron microscopy for high-resolution surface topology and microelectrode or optical sensing for the direct measurement of oxygen and pH gradients are often employed alongside CLSM

V. MECHANISMS LINKING BIOFILM ARCHITECTURE TO ANTIMICROBIAL RESISTANCE

Multiple mechanisms contribute to the antimicrobial recalcitrance of biofilms, and no single mechanism provides a complete explanation. Several architecture-dependent mechanisms act in combination, and their relative contribution varies by organism, antimicrobial agent, and biofilm developmental stage (8). It is important to differentiate between these two related yet distinct concepts. Antimicrobial resistance refers to a stable, heritable trait, typically encoded by mutation or an acquired resistance gene. In contrast, antimicrobial tolerance is a reversible physiological state that allows cells to survive drug exposure without altering their genetically determined susceptibility. Both resistance and tolerance are associated with biofilms, and the architecture described in Section 3 is key for understanding these connections.

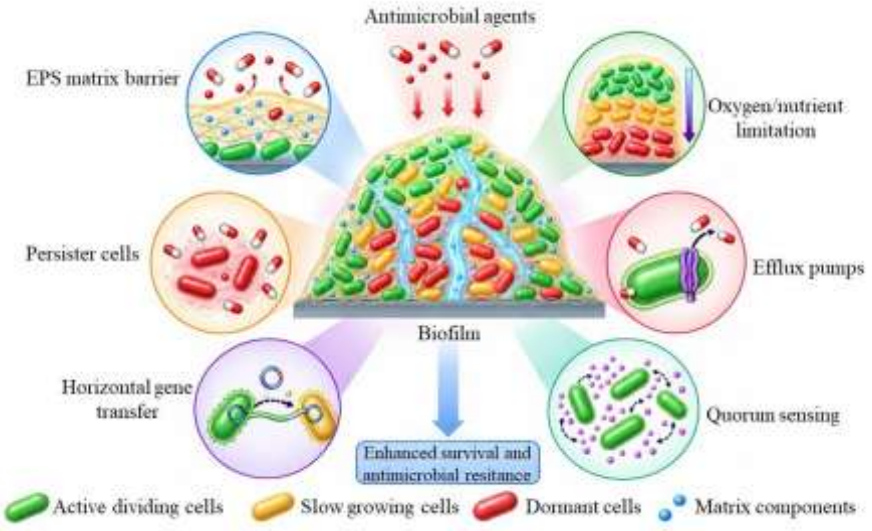

Fig. 3: Mechanism of Antomicrobial resistance in Biofilm (Generated using AI)

5.1 The EPS matrix as a physical and chemical barrier

The simplest and most well-documented mechanism is that the EPS matrix physically prevents antimicrobial drugs from diffusing toward their target cells. The matrix's role as an inert sieving barrier is not the only cause of this process. Instead, antibiotic molecules may interact or chemically bind with matrix components, delaying penetration and lowering the concentration that eventually reaches deeper-lying cells. Furthermore, antibiotics can be actively broken down by matrix-retained enzymes, including released β-lactamases in bacteria like Klebsiella pneumoniae. When these strategies are coupled, they can have a significant therapeutic impact: According to reports, P. aeruginosa biofilms developing on urinary catheters are around a thousand times more resistant to the aminoglycoside tobramycin than genetically identical planktonic cells. It should be noted that matrix-mediated diffusion limitation alone typically does not account for the full extent of biofilm resistance; it should be considered one of several contributing factors rather than the primary explanation (Fig. 3).

5.2 Chemical microenvironment, metabolic heterogeneity, and reduced growth rate

As described in Section 3.2, biofilm architecture induces internal chemical gradients that result in zones with significantly diminished oxygen and nutrient availability, particularly near the biofilm core. Many commonly used antibiotic classes, such as β-lactams, fluoroquinolones, and aminoglycosides, exhibit greater efficacy against actively dividing cells than against slow-growing or non-dividing cells. Consequently, the inherently reduced growth rate of cells within these nutrient- and oxygen-deficient micro-regions imparts a notable degree of antimicrobial tolerance, independent of any genetic change.

5.3 Persister cells and phenotypic tolerance

Persisters are a tiny, unique subpopulation of cells found in biofilms in this varied environment. Genetically similar to their vulnerable neighbours, persister cells go into a temporary, inactive physiological state marked by severely limited macromolecular synthesis and stopped development. Persister cells generally withstand exposure to antibiotics that would otherwise be fatal because the majority of antibiotics target active cellular activities like DNA replication or cell-wall production. This survival is not due to the failure of the drug to reach them but rather because the cellular targets are inactive (9).

Persister cells play a crucial role in the recurrent pattern of biofilm infections, posing significant clinical challenges. Antibiotic treatments can eliminate the metabolically active majority of a biofilm populations while leaving persisters intact, allowing them to repopulate the infection site once treatment is discontinued. Toxin–antitoxin systems, the bacterial stringent response, and SOS DNA-damage response pathways have all been implicated in establishing and maintaining the persister state, although the precise regulatory mechanisms differ across species and remain under investigation.

5.4 Efflux pumps

One well-known resistance mechanism in planktonic bacteria is multidrug efflux pumps, which are transmembrane transporter proteins that actively export antibiotics and other harmful substances from the bacterial cytoplasm. These pumps have been identified as specific genetic determinants of biofilm-associated resistance. The expression of efflux pumps is regulated by quorum-sensing systems in many organisms (Section 5.6). Consequently, enhanced efflux activity within a biofilm is often mechanistically linked to the broader coordination of the biofilm community rather than being independent of it (18).

5.5 Horizontal gene transfer and genetic adaptation within biofilms

In addition to phenotypic tolerance, the biofilm environment actively facilitates the acquisition of genetic resistance. The stabilizing impact of the matrix and the close physical closeness of cells within a biofilm produce favourable circumstances for horizontal gene transfer (HGT) at rates far higher than those usually seen among free-living planktonic bacteria. (10). All three classical HGT mechanisms transformation (uptake of free extracellular DNA), transduction (bacteriophage-mediated gene transfer), and conjugation (direct cell-to-cell transfer via mobile genetic elements such as plasmids and integrative and conjugative elements) have been documented within biofilms, with conjugation considered a particularly important route for the dissemination of antibiotic resistance genes. Recently, membrane vesicles have been identified as an additional route for interspecies gene exchange within biofilm communities.

The clinical significance of this phenomenon is substantial and warrants further investigation. Biofilms function not only as a passive refuge for organisms that are already resistant but also as an active environment capable of generating new resistant strains. Furthermore, they facilitate the dissemination of resistance genes across species boundaries, including between commensal and pathogenic organisms within the same polymicrobial community (19).

5.6 Quorum-sensing regulation of resistance-associated phenotypes

Numerous mechanisms previously described are coordinated rather than incidental, with much of this coordination being facilitated by quorum sensing (QS). QS

is a communication system dependent on cell density, wherein bacteria produce, release, and detect small diffusible signalling molecules to synchronize their collective behaviour across the population[18]. Gram-negative bacteria generally utilize acyl-homoserine lactones (AHLs) for signalling, whereas gram-positive bacteria employ autoinducing peptides (AIPs). Despite the differences in the signalling molecules, both systems result in broadly similar downstream outcomes.

QS regulates transcription of genes governing EPS matrix synthesis, biofilm formation itself, virulence factor production, and, in a number of organisms, efflux pump expression. Given that QS is positioned upstream of many resistance-relevant processes, it has emerged as a promising therapeutic target. Disruption of QS signalling, a strategy referred to as quorum quenching, can reduce EPS production and biofilm formation without directly killing the bacteria. This method may exert less selective pressure for the development of resistance than conventional bactericidal antibiotics. Further, this approach is discussed in Section-7.

5.7 Polymicrobial interactions

Polymicrobial biofilms, rather than single-species communities, are of greater clinical significance, as interactions among co-existing species can enhance antimicrobial resistance in ways that single-species laboratory models cannot replicate. In such biofilms, susceptible species may be shielded physically or chemically by their resistant neighbours. HGT across species can facilitate the exchange of resistance determinants among unrelated organisms within the same matrix. QS signalling can also transcend species boundaries, complicating the diagnosis and treatment of these infections. Polymicrobial dynamics are particularly well- documented in cystic fibrosis lung infections, chronic wound infections, and infections associated with medical devices, where multiple organisms frequently coexist within a single biofilm community.

VI. CLINICAL SIGNIFICANCE OF BIOFILM-ASSOCIATED ANTIMICROBIAL RESISTANCE

The mechanisms previously described are not mere laboratory curiosities; they form the basis of a significant proportion of infections observed in routine clinical practice. Biofilms are involved in approximately 65% of microbial infections and up to 80% of chronic infections in humans (5). They repeatedly appear as central themes across distinct and high-burden clinical contexts (6,7).

Direct biofilm pathology is exemplified by infections linked to medical devices. Catheters, prosthetic joints, heart valves, and pacemakers are examples of devices that provide abiotic surfaces that allow organisms like Staphylococcus epidermidis, Staphylococcus aureus, and other gram-negative bacilli to build biofilms. When antibiotic therapy is not enough to eradicate these infections, the device must often be surgically removed.

Cystic fibrosis (CF) provides one of the best-studied non-device-related examples. Patients with CF produce abnormally viscous airway mucus that predisposes them to infection and provides an environment favouring biofilm formation. This is particularly evident in the mucoid phenotype of *Pseudomonas aeruginosa*, which establishes chronic, biofilm-based lung infection that is rarely eradicated once established (20).

Chronic, non-healing wounds constitute a significant clinical challenge, with biofilm formation increasingly identified as a key factor in the delayed healing. This is distinct from acute wound infections and necessitates different diagnostic and management strategies, such as debridement combined with biofilm-targeted antimicrobial treatments. Biofilms are also heavily implicated in periodontitis, chronic dental plaque-related diseases, chronic rhinosinusitis, otitis media, infective endocarditis and a range of other chronic and recurrent infections.

Given that the six bacterial pathogens contributing most heavily to global AMR mortality; *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* are all well-documented biofilm formers (3,4); the overlap between biofilm biology and the broader AMR crisis described in Section 1 is not incidental but structural. When it comes to infections that are the main cause of resistance-associated mortality, strategies that do not take biofilm architecture into account are probably going to be consistently ineffectual.

VII. THERAPEUTIC AND ANTI-BIOFILM STRATEGIES TARGETING BIOFILM ARCHITECTURE

Even when the causal species is categorized as "susceptible" by routine laboratory testing, conventional antibiotics, which were created and tested primarily against planktonic bacteria, frequently show lower efficiency against biofilm-embedded organisms. Due to this restriction, there is now more interest in tactics that specifically target biofilm architecture, either as stand-alone methods or as supplements to antibiotics (21).

7.1 Quorum-sensing inhibitors and quorum quenching

EPS synthesis, biofilm formation, and other resistance-associated phenotypes are all coordinated by QS (Section 5.6). As a result, interfering with QS signalling has become a viable treatment approach (fig.4). In laboratory and early clinical trials, quorum-sensing inhibitors, such as furanones and structural analogues of AHL signalling molecules, have shown the capacity to lower biofilm development and virulence factor expression. QS inhibitors are typically studied in conjunction with traditional antibiotics because they do not directly kill bacteria. Several studies indicate that this strategy can result in a synergistic antibacterial impact while perhaps lowering the antibiotic dose needed for efficacy. (18).

7.2 Matrix-degrading enzymes

Using enzymes that break down particular EPS components, such as DNase (which targets eDNA), glycoside hydrolases (which target exopolysaccharide), and dispersin B, is an architectural technique that directly addresses the physical matrix. These enzymes have the ability to break down or dissolve the matrix, which causes sessile cells to separate (fig.4). This procedure is essential because it increases the accessibility of implanted cells to host immune systems and improves the penetration of co-administered medications (14,21).

7.3 Nanotechnology-based delivery systems

With physicochemical characteristics like small size, tuneable surface charge, and functionalization potential that enable them to more successfully penetrate the EPS matrix than free antibiotic molecules, nanoparticle-based delivery systems have become one of the most active areas of anti-biofilm research (22, 23). While liposomes, polymeric nanoparticles, nanogels, and metal-organic frameworks enable regulated, localized drug release, metal and metal-oxide nanoparticles may also have inherent antibacterial or matrix-degrading catalytic activity (fig.4). Despite this promise, the clinical translation of nanoparticle-based systems is still restricted due to issues with large-scale manufacturing, the lack of established regulatory pathways for many novel formulations, and incomplete characterization of nanoparticle biodistribution and long-term safety in humans. These issues are covered in more detail in Section- 8 (24).

7.4 Bacteriophage therapy and phage-derived enzymes

Bacteriophages, viruses that infect and lyse specific bacterial species and their derived enzymes represent a further architecturally targeted strategy. Antibiotic penetration can be enhanced, biofilm structure can be disrupted, and EPS matrix components can be directly broken down by phage-encoded depolymerases and endolysins. When phages are used in combination with matrix-degrading enzymes or traditional antibiotics, the results are more effective than when each strategy is used alone (25). Phage therapy is of particular interest for infections caused by multidrug-resistant pathogens for which few effective antibiotic options remain, although practical challenges, including bacterial resistance to phages themselves, unresolved regulatory pathways, and the need for rapid phage susceptibility testing, continue to limit routine clinical use (fig.4).

7.5 Combination and multi-targeted strategies

As no single mechanism can entirely explain biofilm-associated resistance, most researchers now prefer combination and multi-targeted strategies over the use of a solitary anti-biofilm agent. For instance, they may pair quorum-sensing inhibitors, efflux pump inhibitors, or matrix-degrading enzymes with conventional antibiotics, or combine nanoparticle carriers with antimicrobial peptides to improve peptide stability in vivo (21,23). This tactical change reflects a more comprehensive realization that biofilm-associated resistance comes from community behaviour and architecture rather than from a particular druggable target. This tactical change reflects a more comprehensive realization that biofilm-associated resistance comes from community behaviour and architecture rather than from a particular druggable target (fig.4).

VIII. CURRENT CHALLENGES AND FUTURE DIRECTIONS

Numerous obstacles still exist in the practical use of antibiofilm methods, despite notable advances in research. First, because biofilm resistance mechanisms differ significantly between species, infection sites, and host immune environments, a strategy that works well against one organism or type of infection may not work well against another. This suggests that customized, infection-specific approaches are preferable to general anti-biofilm solutions (21). Second, the translation of promising laboratory findings into clinical practice is hampered by the absence of standardized, clinically meaningful biofilm models both in vivo and in vitro.

Third, there are still significant translational obstacles, particularly for methods based on nanomedicine. Despite proven laboratory efficacy, clinical adoption is hampered by large-scale, repeatable manufacturing, incomplete characterization of nanoparticle biodistribution and the biomolecular corona that forms on nanoparticle surfaces in vivo, and unresolved regulatory pathways for many novel formulations (24).

Looking forward, several converging directions are likely to shape the field. These include computational and data-

driven approaches aimed to accelerate the discovery of anti-biofilm compounds and optimize combination regimens. Additionally, stimuli-responsive nanotherapeutics are being designed to release their payload in response to the specific pH, enzymatic, or redox conditions found within a biofilm, improving precision and minimizing off-target effects; and continued development of engineered phage and enzyme-based therapeutics tailored to specific pathogens and matrix compositions (23,24,25). Given the close mechanistic relationship between biofilm formation and antimicrobial resistance described throughout this review, integrating biofilm-aware diagnostics and antimicrobial stewardship into routine clinical practice is likely to prove as important as any single new therapeutic agent.

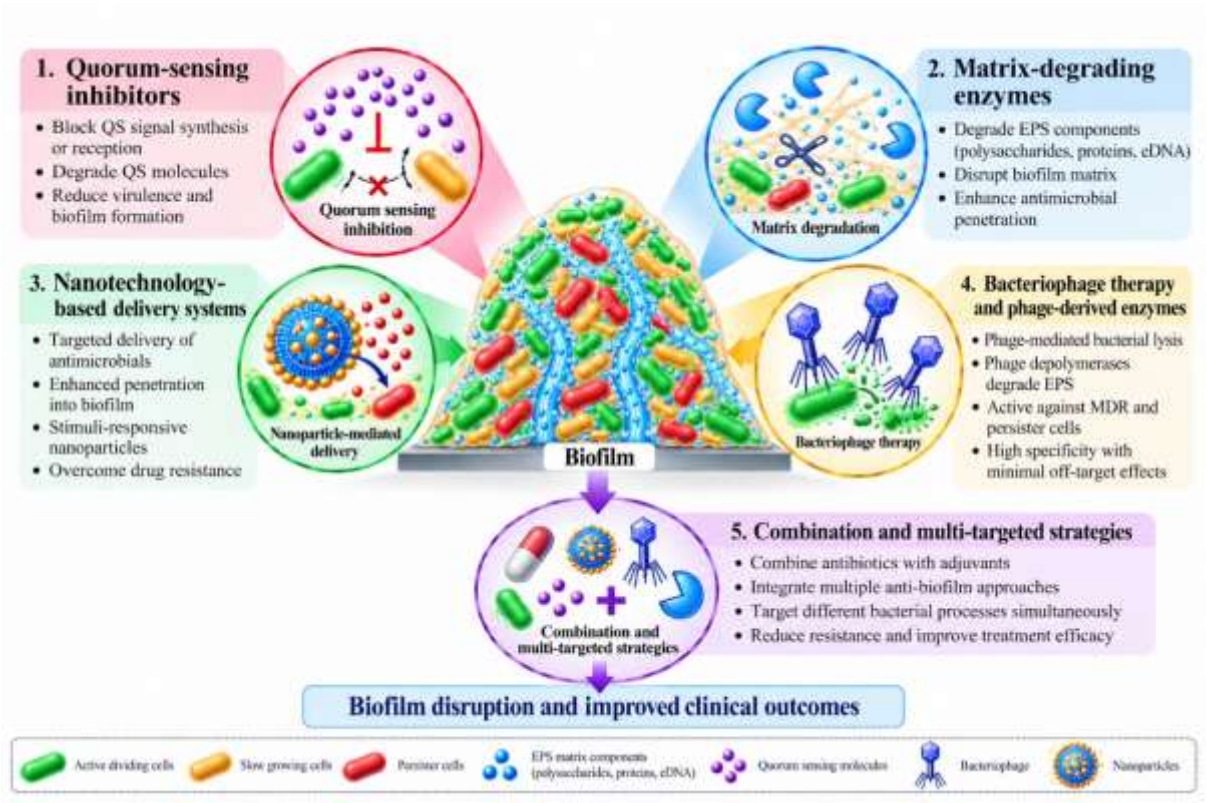

Fig. 4: Anti Biofilm strategies mapped to their targets (Generated using AI)

IX. CONCLUSION

Biofilm architecture is a fundamental and active contributor to antimicrobial resistance and tolerance in chronic bacterial infections, functioning through multiple converging mechanisms rather than singular pathways. The EPS matrix restricts and chemically neutralizes antimicrobial agents. Additionally, the chemical microenvironments generated by these cells induce slow-growing and dormant states, which are inherently challenging to eradicate. Additionally, biofilms' physical proximity speeds up the transfer of resistance genes between cells and even between different species. Therapeutic approaches will probably need to specifically target biofilm architecture because these processes are reliant on structural components rather than specific resistance genes. This strategy could entail providing chemicals that can get past the biofilm's physical defences, upsetting the matrix, or interfering with quorum-sensing coordination. Instead than being used in place of traditional antibiotic therapy, these tactics should be used in conjunction with it. Understanding and directly addressing biofilm architecture will continue to be essential to the larger endeavour to maintain the efficacy of antimicrobial therapy as the burden of antibiotic resistance increases globally.

REFERENCES

[1] Di Martino P. Extracellular polymeric substances, a key element in understanding biofilm phenotype. AIMS Microbiol 2018; 4(2):274-288.

[2] Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE et al. Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050. Lancet 2024; 404(10459):1199-1226.

[3] Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 2022; 399(10325):629-655.

[4] World Health Organization. WHO Bacterial Priority Pathogens List 2024: bacterial pathogens of public health

importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: World Health Organization; 2024.

[5] Davies D. Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov 2003; 2(2):114-122.

[6] Mihai MM, Holban AM, Giurcaneanu C, Popa LG, Oanea RM, Lazar V et al. Microbial biofilms: impact on the pathogenesis of periodontitis, cystic fibrosis, chronic wounds and medical device-related infections. Curr Top Med Chem 2015; 15(16):1552-1576.

[7] Vestby LK, Grønseth T, Simm R, Nesse LL. Bacterial biofilm and its role in the pathogenesis of disease. Antibiotics (Basel) 2020; 9(2):59.

[8] Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev 2017; 41(3):276-301.

[9] Liu HY, Prentice EL, Webber MA. Mechanisms of antimicrobial resistance in biofilms. npj Antimicrob Resist 2024; 2(1):27.

[10] Michaelis C, Grohmann E. Horizontal gene transfer of antibiotic resistance genes in biofilms. Antibiotics (Basel) 2023; 12(2):328.

[11] Wang X, Liu M, Yu C, Li J, Zhou X. Biofilm formation: mechanistic insights and therapeutic targets. Mol Biomed 2023; 4(1):49.

[12] Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol 2010; 8(9):623-633.

[13] Balducci E, Papi F, Capialbi DE, Del Bino L. Polysaccharides' structures and functions in biofilm architecture of antimicrobial-resistant (AMR) pathogens. Int J Mol Sci 2023; 24(4):4030.

[14] Campoccia D, Montanaro L, Arciola CR. Extracellular DNA (eDNA): a major ubiquitous element of the bacterial biofilm architecture. Int J Mol Sci 2021; 22(16):9100.

[15] Bottura B, McConnell G, Florek LC, Smiley MK, Martin R, Foylan S et al. Oxygen microenvironments in Escherichia coli biofilm nutrient transport channels: insights from complementary sensing approaches. Microbiology (Reading) 2025; 171(5):001543.

[16] Moorthy RK, Casey E. Modeling multiscale architecture of biofilm extracellular matrix and its role in oxygen transport. Biotechnology and Bioengineering. 2025 Oct 22.

[17] Reichhardt C, Parsek MR. Confocal laser scanning microscopy for analysis of Pseudomonas aeruginosa biofilm architecture and matrix localization. Front Microbiol 2019; 10:677.

[18] Zhao X, Yu Z, Ding T. Quorum-sensing regulation of antimicrobial resistance in bacteria. Microorganisms 2020; 8(3):425.

[19] Cui S, Kim E. Quorum sensing and antibiotic resistance in polymicrobial infections. Commun Integr Biol 2024; 17(1):2415598.

[20] Høiby N, Ciofu O, Bjarnsholt T. Pseudomonas aeruginosa biofilms in cystic fibrosis. Future Microbiol 2010; 5(11):1663-1674.

[21] Abdelhamid AG, Yousef AE. Combating bacterial biofilms: current and emerging antibiofilm strategies for treating persistent infections. Antibiotics (Basel) 2023; 12(6):1005.

[22] Joshi AA, Patil RH. Nanoparticles targeting biofilms: a new era in combating antimicrobial resistance. Med Microecol 2025; 26:100156.

[23] Xie Y, Liu H, Teng Z, Ma J, Liu G. Nanomaterial-enabled anti-biofilm strategies: new opportunities for treatment of bacterial infections. Nanoscale 2025; 17(10):5605-5628.

[24] Farah H, Kadhim-Abosaoda M, Mohaisen-Mousa H, Renuka Jyothi S, Priyadarshini-Nayak P, Bethanney Janney J, Singh G, Singh-Chauhan A, Kumar-Mishra M. Nanomedicine strategies against biofilm-associated infections: advances, challenges, and translational barriers. MicrobiologyOpen. 2026 Feb;15(1):e70210.

[25] Husain FM, Zahra A, Ali A, Kamthan M, Al-Shabib NA, Farooqui Z et al. Bacteriophages and their enzymes: allies against microbial biofilms. Pharmaceuticals (Basel) 2025; 18(12):1771.