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Unveiling An Antibiofilm Marvel to Combat Antibiotics Resistance

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Abstract:
Creative approaches are essential to fight against bacterial infections while the worldwide issue of antibiotic resistance keeps growing. To combat the persistent biofilms that bacteria create, which are a major reason for treatment failure, this study presents a novel Antibiofilm Marvel (ABM). We provide an in-depth analysis of the makeup and mode of action of ABM, demonstrating its capacity to rupture and destroy biofilms, increasing the sensitivity of bacteria to traditional antibiotics. Furthermore, the work demonstrates the synergistic impact of ABM when used with currently available antibiotics, leading to a significant recovery in antibacterial efficacy. The present work highlights the need to prioritize biofilm-targeted strategy in the battle against antibiotic resistance and provides a possible path forward for the development of next-generation antibacterial medicines. An important turning point was the introduction of ABM. This review article is focusing on the various antibiofilm agent (synthetic, chelating agents, and antibiotics) along with their mechanisms of inhibition (AHL-mediated quorum sensing inhibition, Extracellular Polysaccharide Substance of Biofilm Dispersion by Enzymes, Inhibition of biofilm by polysaccharides, Inhibition of cell division and survival, Cleavage of peptidoglycan, Inhibition of c-di-GMP signaling system, Neutralization or disassembly of lipopolysaccharides) This article updates the recent findings on the other active antibiofilm agents.

Keywords: Biofilms, Anti-biofilm agent, Biofilm models, Acyl Homo-serine Lactone, Lipopolysaccharides (LPS), Quorum sensing.

Introduction

It is quite likely that pathogenic bacterial or fungal species are present in biofilms. These may target receivers who are immunocompromised, among other people. These infections can also cause food poisoning (S. aureus, Bacillus cereus) or contribute to disorders like gastroenteritis (Escherichia coli, S. enterica). The increased durability of biofilms to chemical disinfection, human immunological response, and anti-microbial treatment presents another difficulty. Because of all these difficulties, biofilms are becoming a major worry in clinical, industrial, and environmental settings. Despite much investigation, no antibiotic or antimicrobial agent has been created to date to remove or treat biofilms [1,2]. Given this,

scientists throughout the world are left with little choice but to find novel ways to prevent the growth of biofilm or to create a more advanced class of natural antibiotics. In this regard, it is well known that phytochemicals exhibit anti-microbial and anti-biofilm properties against a wide range of pathogenic species. Because of their multi-targeted nature, they may be crucial in slowing the emergence of drug resistance [3]. The purpose of this study was to determine if sulfated polysaccharides derived from green algae could effectively combat biofilms generated by Vibrio harveyi and Salmonella enterica. By using the agar cup diffusion assay with increased concentrations of Cr-SPs, it was demonstrated in this study that Cr-SPs exhibited effective anti-microbial activity against both S. enterica and V. harveyi.

There was a gradual increase in the clear zones, indicating that Cr-SPs are effectively inhibiting the growth of both S. enterica and V. harveyi. Furthermore, the time-kill experiment demonstrated that beginning at 3 hours, bacterial growth was gradually reduced with increasing doses of Chlamydomonas Reinhardtian (Cr-SPs), and total growth suppression was shown from 12 hours to 48 hours respectively [4].

The term "biofilm" describes complex communities of microorganisms that are tightly embedded in an extracellular matrix (ECM) and can be found hanging to a surface or forming aggregates without adhering to a surface, as shown in Pseudomonas aeruginosa, Staphylococcus aureus, and some other bacteria [5-7]. The bacteria can survive harsh environmental circumstances including hunger and desiccation because of their biofilm lifestyle, which also enables them to cause a variety of chronic illnesses. For patients with weakened immune systems, it is thus thought to be a significant contributor to recurring nosocomial infections [8, 9]. Approximately half of all nosocomial infections are limited to patients who have indwelling medical devices, such as cardiac pacemakers, joint prostheses, contact lenses, dentures, and prosthetic heart valves [10,11]. Bacterial cells may attach themselves perfectly to these foreign body surfaces. Consequently, the presence of implants has been linked to a notable increase in biofilm production [12]. Many times, using antibiotics such as imipenem, colistin, and others only results in a reduction of the biofilms—they cannot completely eradicate the biofilm. It is impossible to achieve the minimum concentration of antibiotics in vivo due to their toxicity and adverse effects. Antibiotic therapy has consequently become less effective due to the elevated minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for the biofilm bacterial cells [13–15].

Additionally, biofilms shield invasive bacteria from the host's immune system by preventing phagocyte and complement system activation [16–18]. They also boost the bacterium's resistance to standard antibiotics by a factor of 1000 [19-24]. This antimicrobial tolerance could be explained by a few more reasons. Previous experiments have identified several causes for the resistance, including the composition and structure of the biofilm, the availability of nutrients and oxygen to the bacterial cells, and the presence of both innate and acquired bacterial resistance. A study on P. aeruginosa revealed the role of biofilm in resistance, as the mucoid structure of the biofilm was found to be responsible for high resistance against tobramycin [25].

Another possible cause of antibiotic resistance in biofilm-associated bacteria is their metabolic status. Because they divide so seldom, bacteria in the nutrient-depleted zones of the biofilm may enter a dormant condition similar to that of the stationary phase, rendering them resistant to antibiotics [26, 27]. Because dividing cells are susceptible to some antibiotics, such as beta-lactams, they should not be used. According to Walters et al., P. aeruginosa showed evidence of antibiotic resistance in the presence of restricted oxygen supply, with antibiotics only working at the air-biofilm interface (the portion of the biofilm exposed to oxygen, which is between 50 and 90 μm in length) [28]. Furthermore, research shows that when biofilm is exposed to a sub-lethal concentration of an antibiotic, the rate of mutation in biofilm cells

is higher than in planktonic counterparts, which increases the efficiency of plasmid (including antibiotic resistance gene) transfer by ten times [29].

❖ Process of Biofilm Formation

On every surface, the production of biofilms primarily occurs in three phases. Cells adhere to a surface in the first stage, assemble to create microcolonies, and then differentiate into a mature structure called a biofilm. Both mechanical and active techniques are used to disassemble or disperse biofilm once it has finished developing [31]. While Lifshitz–Van der Waals, acid–base, hydrophobic, and electrostatic contact forces control bacterial adherence to the substratum, sedimentation, Brownian motion, and hydrodynamic forces specifically influence bacterial deposition [32]. The development of biofilms, especially during the early attachment phases, is aided by some surface-related proteins, including Outer membrane protein A (OmpA), fibronectin-binding proteins, protein A, SasG, biofilm-associated protein (BAP), and several other elements [33-37].

Certain species may anchor themselves to the matrix or to the previous colonies directly, but they are unable to adhere to a surface. Cell-cell communication networks work with small signaling molecules to facilitate this invasion. Most people refer to this phenomenon as "quorum sensing" [38,39]. Quorum-sensing controlled phenotypes include biofilm development [40]. Bacterial cells in biofilms are encased in an extracellular matrix, a complex combination of highly polar biomolecules comprising proteins, polysaccharides, lipids, and nucleic acids [41].

The matrix offers defense against antimicrobial exposure and immune cell assault, among other stressors. But the antibacterial ingredient is not mechanically protected by the biofilm's matrix. This was verified by a study that demonstrated ampicillin could penetrate the biofilm formed by a K. pneumoniae strain lacking β-lactamase, while ampicillin was unable to do so in a wild-type strain of the bacteria that had β-lactamase [42]. This suggests that in the latter case, ampicillin was quickly broken down by β-lactamase prior to infiltrating the wild type biofilm. As soon as the bacteria begin to secrete extracellular polysaccharide material (EPS), the second, irreversible stage of biofilm creation begins. Up to the third stage of development, EPS is continuously secreted, guaranteeing that bacteria can safely adhere to the surface inside of a densely packed biomolecular layer [43].

The fully developed biofilm now has a three-dimensional, tower-like structure. These towers are made up of tiny channels that transport waste, water, and nutrients. The planktonic bacteria are housed in the towers' tiny cavities. Studies also show that various bacteria have quite varied biofilm architectures and organizational systems. The precise cause of this mutation is yet unknown. On the other hand, exopolysaccharides Pel and Psl control the development of biofilms in other pseudomonads, such as P. aeruginosa [47–49], whereas the adhesive protein LapA controls the formation of biofilms in P. putida [44–46].

Therefore, differences in the extracellular matrix (ECM) component may be the cause of the variations in biofilm structure. Eventually, the holes holding bacteria that are not adhered to the surface are emptied when these towers either erode (small portions) or are sloughed off (big areas) and become detached. The discharge of new germs into the environment comes next [50, 51]. Increases in c-di-GMP levels, an intracellular secondary messenger, signal the start of biofilm formation and virulence in a number of bacterial species, including Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens, Yersinia pestis, Escherichia coli, Vibrio cholerae, Burkholderia cenocepacia, Salmonella enterica, Clostridium difficle, Klebsiella pneumoniae, Vibrio cholerae, and Bacillus subtilis [44, 45, 52-60]. c-di-GMP was first discovered as a distinct secondary messenger upon allosteric activation of cellulose

synthase in Gluconacetobacter xylinus [59]. By attaching to a variety of receptors, such as enzymes, adaptor proteins, transcription factors, and riboswitches, c-di-GMP performs its role [62]. Different c-di-GMP circuits include the participation of various bacterially produced phosphodiesterases and diguanylate cyclases [63]. Additionally, it has been documented that a variety of environmental stimuli and transducer processes raise the cell's cdi-GMP level. This promotes the synthesis of adhesins and facilitates the extracellular matrix's secretion [64, 65]. The formation of extracellular matrix components in P. aeruginosa, including CdrA adhesin, alginate exopolysaccharide, Pel, and Psl, is favorably regulated by the amount of c-di-GMP [55, 66]. Small regulatory RNAs (sRNA) also control the development of biofilm in a number of bacterial species in addition to c-di-GMP [67].

❖ Models to Study Biofilms

Biofilm model study: The understanding of biofilm biology is improved by the study of many biofilm model systems. Both in-vivo and in-vitro model systems are used to study the biofilms. There are three main categories of in-vitro biofilm model systems: microcosms, open or dynamic models, and closed or static models. Microtitre plate-based closed model systems are the most widely used closed model systems which employ batch and static growth conditions,[68]. Because there is no movement of media, product, or waste materials into or out of the reactor in this model, the experimental circumstances in the wells progressively change. For example, signalling components accumulate, the bacterial population grows, and the medium's nutrients eventually run out.

Many tests may be run simultaneously because it is affordable and just requires a modest number of reagents [69]. Furthermore, biofilm deficient mutants and biofilm-forming wild type strains can be distinguished using microtitre plate-based models [70, 71], the antimicrobial and anti-biofilm effects of various antimicrobial compounds can be ascertained, and biofilm initiation factors, including adhesins, pili, flagella, enzymes involved in cyclic-di-GMP metabolism, and genes responsible for extracellular polysaccharide production, can be identified [72, 73].

The flow displacement biofilm model is the most widely used open and dynamic model for studying biofilms. In this model system, addition and release of nutrients and waste products can happen, in contrast to the microtitre plate approach [68, 74]. Since it closely mimics in-vivo circumstances, the dynamic model of biofilm development utilizing perfused silicone tubes is one of the most useful models for investigating biofilms. In a silicone tube system, biofilms are created under dynamic conditions, and then the tube is broken into small pieces for additional research and treatment [75].

Another in-vitro model system for investigating biofilms that replicate in-situ conditions in a controlled setting is the microcosm [76–78]. This system is used to examine dental, wound, oral, and stream biofilms. By utilizing the same medium and constructing an artificial environment, it is possible to transform both in-vitro and in-vivo systems into microcosms in order to evaluate the behavior and metabolism of the cells. In addition, there is an ex-vivo model system that works with organs and tissues taken from living things for additional research and testing in a lab setting. This approach can be helpful in tracking the colonization and spread of germs within a certain tissue or organ. There should be some in-vivo model system research conducted to validate the simplified results that the in-vitro model studies produced.

Mammalian 8 models that are closer to humans must be studied to address a variety of therapeutic and diagnostic issues. The primary topics of investigation for these tissue-associated model systems include wound infections, urinary tract infections, and lung infections [72, 79]. The study of these infections has made use of a variety of other models, including those for central venous catheters, subcutaneous foreign

body infections, intraperitoneal foreign body infections, urinary tract infections, infections of the ear, nose, and throat, respiratory tract infections, and osteomyelitis infections [72]. Due to some challenges with using mammalian models, researchers have shifted to using non-mammalian model systems, such as Danio rerio, Drosophila melanogaster, and Caenorhabditis elegans [80].
These model's shorter generating times and cheaper costs are their main advantages. Furthermore, because of their tiny sizes, they are simple to maintain in microtitre plates, which facilitates high throughput screening for the development of biofilms.

❖ Anti-biofilm agents and their mechanism of action

The term "anti-biofilm molecules" refers to a group of compounds that have been identified as having the ability to inhibit the formation of biofilms. These compounds have been primarily isolated from natural sources [108], but they can also be synthetic, chelating agents, and antibiotics. The various anti-biofilm molecules and the microorganisms they target are listed in table 1. The various anti-biofilm molecules, along with their mechanisms of inhibition, are listed in table 2.

Table-1: antibiofilm agents and their target

S.No.	Antibiofilm agents	Sources	Their targets	References
1.	Epigallocatechin gallate (EGCG)	Camellia sinesis (Green tea)	Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli	154
2.	Ellagic acid	Camellia sinesis	Streptococcus dysgalactia	149
3.	Reserpine	Rauwolfia vomitoria, Rauwolfia serpentine	Klebsiella pneumoniae	155
4.	Polymyxin B	-	P. aeruginosa, S. aureus, E. coli	157
5.	Berberine	Berberis aquifolium, B. vulgaris, B. aristata	K. pneumoniae	155
6	Chitosan	Chitin	K. pneumoniae	155
7	Eugenol	Ocimum plants, Syzigium aromaticum	K. pneumoniae	155,156
8	Curcumin	Curcuma longa	K. pneumoniae	155
9	**Lantibiotics:** Nisin Subtilin Epidermin	Lactococcus lactis B. subtilis strain ATCC6633 Staphylococcus epidermidis Tu3298	S. aureus, Staphylococcus epidermis Lactococcus lactis Lactococcus lactis	159,160

	Gallidermin	Staphylococcus gallinarum Tu3928	S. aureus S. epidermidis	159,160
10	**Antimicrobial peptide (AMP):** LL-37	Human cationic host defense peptide	P. aeruginosa, S. aureus, E. coli	157,158, 164-166
11	Lytic peptide (PTP-7)	Synthetic analogue from Gaegurin 5	P. aeruginosa, S. aureus, E. coli	157,158, 164-166
12	Sushi peptides	Derived from sushi-3 domain of Factor C, which is a LPS-sensitive serine protease of horseshoe crab coagulation cascade	P. aeruginosa, S. aureus, E. coli	157,158, 164-166
13	**Chelating agents:** (a)Sodium citrate (b)Tetrasodium EDTA (c) Disodium EDTA	-	Staphylococcus species, P. aeruginosa	158
14	**Enzymes:** Deoxyribonuclease I, glycoside hydrolase (dispersin B)	-	Staphylococcus and Enterococcus	158,177
15	Silver	-	P. aeruginosa, S. proteamaculans	109

Table 2- Mechanisms of action of different Anti-biofilm agents

S.No.	Mechanism of action	Agents associated	References
1	Cleavage of peptidoglycan	Tannic acid, Endolysins (PlyC), Epigallocatechin gallate (EGCG)	123, 127, 136
2	Biofilm disassembly	A cyclic autoinducing peptide (AIP), Nuclease, extracellular proteases (eg. sarA, sigB, Esp), antiamyloid molecules (AA-861, parthenolides), D-Tyrosine, Ethyl-pyruvate	75, 171, 172, 174
3	Neutralization/disaggregation of LPS	Polymyxin (B and E), Gramicidin S, Sushi peptides, PMAP-23	118, 121, 158
4	Inhibition of AHL-mediated quorum sensing pathway	Halogenated furanone compounds, Quercetin	81

5	Inhibition of (p)ppGpp regulated stringent response	Peptide-1018, Peptide-1038	175, 176
6	Dispersion of Extracellular Polymeric Substance (EPS) of biofilm	Deoxyribonuclease I and glycoside hydrolase dispersin B	157
7	Alteration of membrane permeabilization	Lantibiotics (Nisin, gallidermin), Lytic peptides (PTP-7), Sophorolipids, Polyhexamethylene biguanide, Chlorhexidine, Penta silver hexaoxoiodate	120, 161,162,163
8	Inhibition of cell division or cell survival	Pyrrhocoricin, Microcin B17	110,114
9	Inhibition of macromolecule synthesis and adhesion of cells	Buforin II, PR-39, Indolicidin, LL-37, Bacteriocins, Cadexomer iodine, Mannosides, Pilicides	164-170
10	Inhibition of biofilm by polysaccharides	EPS273, Psl and Pel, K2, PAM galactan, A101, PslG, Polysaccharides of algae, plants and animals	96,97,99,100,102,107
11	Inhibition of c-di-GMP signaling system	LP 3134, LP 3145, LP 4010, LP 1062, ebselen, ebselen oxide Desformylflustra bromine	139,140
12	Inhibition of curli biosynthesis	Analogs of FN075 and BibC6 of ring-fused 2- pyridones	132

1. AHL-mediated quorum sensing inhibition

Quorum sensing mediated by AHL inhibition During quorum sensing by many bacteria, particularly Gram-negative bacteria, employ N-acyl homo-serine lactones (AHLs) as signaling molecules to regulate their population density and promote motility during swarming. The lengths of these signaling molecules vary, and they are produced by a LuxI-type synthase with alterations on the acyl side chains [81]. The target gene's expression is regulated when these chemicals bind to a corresponding LuxR-type transcriptional activator protein at certain critical concentrations [82, 83]. The Australian macroalga Dilsea pulchra produces natural furanone, which is the source of the synthetic halogenated furanone chemical, a secondary metabolite derivative. This substance has the power to obstruct bacterial signaling pathways and swarm cell motility. Additionally, it was proposed that the structural similarity between AHL molecules and D. pulchra furanones influences how a putative regulatory protein interacts with AHL molecules by binding to the receptor in a competitive manner. At ecologically relevant concentrations, furanones suppress surface aggregation characteristics in bacteria that are relevant to the environment [84]. Furanone 56 inhibits the quorum sensing-regulated transcription of lasB-gfp (ASV) reporter fusion by reducing extracellular chitinase and elastase activity, while having almost little effect on bacterial growth or protein synthesis.

According to studies, furanone affects the regulation of genes linked to quorum sensing biofilm maturity by targeting the Ahl system, which is involved in quorum 12 sensing, and by penetrating P. aeruginosa's biofilm matrix. This chemical modifies the biofilm's structure, accelerating the pace at which bacteria detach from the substrate and causing the bacterium's biomass to be lost [81]. Furthermore, it was shown that furanone causes the displacement of AHL molecules from Lux R, indicating that furanone is competent to bind to the appropriate AHL signal for the LuxR receptor site.

Currently, a number of experimental findings corroborate the findings regarding furanones. These include the suppression of bioluminescence expressed in response to AHL [85], the inhibition of the pathogenesis and synthesis of virulence factors controlled by AHL [81, 86], and the inhibition of luminescence regulated by quorum sensing [87].

Certain polyphenols, such as ellagic acid, tannic acid, and EGCG, are thought to work through a similar mechanism to prevent the formation of biofilms; however, because they are less effective than furanones, larger concentrations of these polyphenols are needed [40].

Flavonoid functions as an anti-biofilm agent against S. aureus via influencing quorum sensing as well. It causes a concentration-dependent inhibition of alginate synthesis, which lowers adhesion during the development of biofilms. Additionally, it induces swarming movement and decreases the formation of exopolysaccharides (EPS), which are necessary for the first adhesion of bacteria [88]. In addition to quercetin, two more synthetic flavanoids have been discovered that have the ability to function as antibacterial agents against S. aureus biofilm and scattered cells [89].

According to a few other investigations, usinic acid may also have an inhibitory impact on the S. aureus biofilm and modify the shape of the P. aeruginosa biofilm. It is yet unknown exactly how exactly this interferes with quorum sensing, however researchers have speculated that this could be the case [90]. Curcumin is a phytochemical derived from the rhizome of Curcuma longa that has a strong antibiofilm impact by modifying the expression of genes linked to swarming motility and alginate formation, as well as quorum sensing [91].

2. Extracellular Polysaccharide Substance of Biofilm Dispersion by Enzymes

The biofilm's Extracellular Polysaccharide Substance (EPS) shields the microbes from several antibiotic substances. These substances would come into contact with the liberated biofilm cells as well as any leftover ones due to the disarray of the EPS. Exo-polysaccharides can be broken down by certain enzymes, including DNases and polysaccharide 14 lyases [92]. Similarly, the two main enzymes that can act as anti-biofilm agents are DNase I and Dispersin B [93, 94]. Extracellular DNA (eDNA) included in the biofilm structure may be broken down by DNase I, while a glycoside hydrolase called Dispersion B breaks down polymers of β1-6 N-acetylglucosamine (PNAG), an extracellular polysaccharide that promotes bacterial aggregation. It can also spread the EPS layers found on medical equipment [143, 144]. When antimicrobial drugs are combined with these biofilm-dispersing enzymes, the bacteria established in the EPS are killed more effectively [95].

3. Inhibition of biofilm by polysaccharides

Biofilms require extracellular polysaccharides as a fundamental component. It has recently been discovered that certain exopolysaccharides exhibit antagonistic effects on the production of biofilms. They can cause the produced biofilm to disperse in addition to inhibiting the production of new biofilms [96, 97]. Exo-polysaccharide EPS273, derived from the marine bacteria P. stutzeri, has been shown in a recent

experiment to inhibit P. aeruginosa biofilm development by targeting virulence factors such as rhamnose, pyocyanin, and exoprotease. By interfering with the synthesis of pyocyanin, which in turn reduces the generation of H2O2, EPS273 eventually prevents the release of eDNA, which is necessary for the development of stable biofilms [98].

Additionally, this molecule is shown to decrease infection associated to biofilm in lung cells and zebrafish embryos. Furthermore, it functions as a strong antioxidant, which lowers superoxide and hydroxy radicals. Therefore, EPS273 can be used to combat P. aeruginosa, which causes nosocomial infections and food spoiling, in both the healthcare and food industries. Studies on its structure showed that EPS273 had traits common to polysaccharides. The main monosaccharide molecules of EPS273 are 35.4% glucosamine, 28.6% rhamnose, 27.2% glucose, and 8.7% mannose. According to HPGPC analysis, this molecule has a molecular weight of 190 kDa [99]. There have also been reports of other different types of anti-biofilm polysaccharides. In dual-species biofilm in-vitro settings, Psl and Pel from P. aeruginosa PAO1 inhibit S. epidermidis' capacity to build biofilm [100, 101]. The E. Coli capsule's K2 polysaccharide and the K. kingae strains' PAM galactan control the biofilm architecture of their colonies by creating water channels or preventing the biofilm from spreading, respectively, suppressing the biofilm in response to their surroundings [102, 103]. P. aeruginosa biofilm is dispersed by another polysaccharide, A101 from V. cholerae QY101 [96]. It has also been found that PAM galactan, an exopolysaccharide from the biofilm of K. kingae, disperses the S. epidermidis biofilm [102].

Only a few of these anti-biofilm polysaccharides can disperse biofilms in their early phases before they reach maturity, but most of them, particularly bacterial ones, exhibit broad-spectrum anti-biofilm action. In industrial and clinical settings, where antibiotic-resistant biofilms are a major cause of a range of nosocomial infections, various oligosaccharides or polysaccharides with antibiofilm capabilities can be employed. They can be used with existing antibiotics to lower the minimum dose needed to eradicate biofilms [96], as an adjuvant to reduce the risk of medical device-related infections [102–105], or as a means of delivering saccharide prebiotics with probiotics [106]. One of the most significant polysaccharides in P. aeruginosa biofilm matrix, Psl, is biosynthesized in part by another protein, PslG. According to studies, endogenous delivery of PslG disperses premade-developed biofilm and prevents biofilm development by targeting the Psl in the matrix. Structural investigation of PslG indicated that it is an endoglycosidase. Studies conducted in vivo have demonstrated that PslG therapy improves biofilms' sensitivity to antimicrobials and the host immune system [107].

4. Inhibition of cell division and survival

The survival of bacteria in biofilms and their subsequent migration to new locations depend heavily on cell division. Silver builds up in intracellular vacuoles, causing damage to the plasma membrane and an adjustment in the electric potential that stops cell division [108, 109]. Certain antimicrobial peptides work by preventing cytoplasmic proteins from doing their job, which is essential for cell division and survival. These peptides enter the bacterial cytoplasm through the flip-flop mechanism or by creating a channel in the protein that makes up the outer membrane. Proline content is high in pyrrhocoricin, apidaecin, and drosocin, three antimicrobial peptides [110,111,112].

All of these peptides can attach to the multi-helical lid region of the bacterial heat shock protein DnaK, which can obstruct the chromosomal DNA replication process at its beginning. Additionally, they obstruct the DnaK-DnaJ interaction, which results in bacterial mortality. Pyrrhocoricin enters the bacterial cytoplasm through the C-terminus, and the N-terminus is in charge of preventing the DnaK protein's

ATPase function from becoming active [110]. Furthermore, proline-rich AMPs aggressively penetrate bacterial cells and obstruct the beginning of translation by binding to the ribosome tunnel [113]. An antibiotic peptide from Enterobacteriaceae called microcin B17 is produced ribosomally and inhibits DNA gyrase, which in turn slows DNA replication. This peptide is also the first to be able to block a type II DNA topoisomerase [114]. In addition, chelating chemicals such as EDTA can strengthen the cell wall, which in turn causes the biofilms to become unstable by retaining iron, zinc, magnesium, and calcium. They are hence appropriate for managing biofilms [115]. Because it is cationic, the natural polymer chitosan may break down negatively charged cell membranes as soon as microorganisms attach themselves to the surface [116].

5. Neutralization or disassembly of lipopolysaccharides

A viable substitute for traditional antibiotics, the antimicrobial peptide (AMP) is thought to be a potent anti-biofilm agent. Low molecular weight proteins that have undergone evolutionary conservation and possess antibacterial properties against bacteria, viruses, and fungi are known as AMPs. They may penetrate the lipid bilayer and dissolve in an aquatic environment because they are typically positively charged and have both hydrophilic and hydrophobic sides [117]. Lipopolysaccharides (LPS) and antimicrobial peptides often bond electrostatically through the interaction of two cationic amino acids (lysine and arginine) and their corresponding head groups. The peptide's hydrophobic amino acids and the fatty acyl chains of LPS interact hydrophobically to stabilize the complex [118, 119]. This destabilizes the lipid head groups by causing numerous pore development, which compromises the integrity of the cellular membrane. PTP-7 is a synthetic counterpart of Gaegurin 5, a lytic peptide. Despite being a cationic peptide, large metal ion concentrations, negatively charged extracellular polysaccharides in the biofilm matrix, and acidic pH did not affect its action. Instead, it can efficiently destroy bacteria by penetrating deep into the biofilm [120]. Gram-negative bacteria's outer membrane becomes permeable when polymyxins, particularly polymyxin B (pentabasic decapeptide antibiotic) and polymyxin E (colistin), attach to lipid A of the LPS. Furthermore, Gramicidin S disrupts the integrity of both Gram-positive and Gram-negative bacteria's membranes. The precise targets that these two cationic cyclic peptides have in the cell membrane cause disruptions in the hydrophobic interactions at the enzymes' ligand binding sites. Toxicological improvement, structural analysis, and clinical testing have to be carried out in order to use it in a therapeutic setting [121]. Similarly, the way that sushi peptides, a by-product of Factor C (the horseshoe crab coagulation cascade's LPS-sensitive serine protease), break apart LPS aggregates is similar to that of detergents. They also exhibit LPS-neutralizing properties. With palmitoyl-oleoyl-phosphatidylglycerol (POPG), they have extremely particular effects. The lipid bilayer is rendered more fluid by unsaturated POPG, which also promotes peptide entrance, so entirely upsetting the stability of the membrane [118, 122].

6. Cleavage of peptidoglycan

The majority of bacteria's cell walls contain peptidoglycan, which may be broken down to prevent the formation of biofilms. In Staphylococcus aureus, the polyphenolic substance tannic acid prevents the production of biofilms while having no effect on bacterial growth [123]. It was discovered that the immune-dominant Staphylococcal Antigen A (IsaA), a potential lytic transglycosylase that cleaves peptidoglycan, is responsible for the mechanism of action [124]. The enzymes known as transglycolases resemble lysozymes and are responsible for breaking the β-1,4-glycosidic link that connects N-acetyl

muramic acid (MurNAc) to N-acetyl glucosamine (GlcNAc) [125]. By raising IsaA's extracellular level, tannic acid prevents the development of biofilms [123].
Peptidoglycan cleavage decreases the production of biofilms in a number of ways, including by changing the makeup of the proteins and teichoic acids on the cell wall. Additionally, signaling molecules that might control the expression of genes relevant to biofilms may be released as a result of peptidoglycan breakdown [126]. Bacteriophages are known to encode endolysins, a special type of peptidoglycan hydrolases that break down bacterial cell walls and release bacteriophage offspring [127]. Endolysins often have a species-specific mode of action. In the end, they connect to and break the cell wall, causing hypotonic lysis and bacterial death [128]. Multiple antibiotic-resistant strains can be treated with endolysins. A particular streptococcal bacteriophage endolysin known as PlyC [129–133] works by upsetting the in vitro biofilms.
Understanding the microorganisms causing illnesses is necessary for this bacteriophage treatment since correct design of bacteriophages depends on this knowledge. Another chemical that inhibits bacteria is called epigallocatechin gallate, a polyphenol that binds to peptidoglycan to cause cell wall damage [134, 135]. This interferes with the first docking phase of biofilm formation, which is mostly caused by hydrophobic contacts [136].

7. Inhibition of c-di-GMP signaling system

There are three different types of bacteria: the planktonic state, which causes acute infections and is readily treated with the appropriate dosage of antibiotics; the biofilm state, which causes persistent infections and is more difficult to treat with antibiotics. The scattered state, a discrete step in the transition from the biofilm to the planktonic state, is the third type. Both the internal spread of diseases by biofilms inside hosts and the transfer of germs across hosts are facilitated by the process of dispersion. C-di-GMP, or cyclic di-GMP, is a secondary messenger involved in the production of biofilms. Changes to the c-di GMP signaling pathways in bacteria can affect how biofilms grow and spread in a clinical setting [137]. Bacterial cells reduce the quantity of c-di-GMP under stress situations like famine, nitrosative environments, etc. by activating phosphodiesterase, which causes the biofilm to disperse. This study also shown how the physiology and pathogenic potential of biofilm-dispersed cells differ significantly from those of biofilm and planktonic cells. Because distributed cells express more virulence-related genes at higher levels than biofilm and planktonic cells, they are shown to be more virulent against C. elegans and immune cells. The biofilm-dispersed cells exhibit decreased rsmY and rsmZ expression in addition to a decreased c- 24 di-GMP concentration, which results in poor siderophore synthesis by bacterial species [138]. Through their ability to chelate iron from the surrounding environment, siderophores have been discovered to play a role in preventing the development of biofilms by decreasing the survival of scattered cells. The dispersal-based anti-biofilm action is induced upon chemical administration. The injection of an antimicrobial agent is desired in addition to the dispersing agents since it would prevent the development and spread of the scattered cells, which can evade the macrophage-mediated phagocytosis. It is possible to completely remove the biofilm by combining an iron chelator with the dispersing and antibacterial agents [138]. The small molecules that prevent P. aeruginosa and Acinetobacter baumannii from forming biofilms include LP 3134, LP 3145, LP 4010, and LP 1062. Diguanylate cyclase (DGC) is the enzyme that causes the creation of c-di-GMP. There have been reports that all of these compounds prevent P. aeruginosa from spreading its biofilm. Of them, only two were non-toxic to eukaryotic cells 6, making them viable candidates for biofilm inhibition [139]. Other compounds that underwent tests

including the differential radial capillary action of ligand assay were also shown to be inhibitors of the allosteric binding of c-di-GMP. Administration of ebselen, a synthetic organoselenium medication, decreased DGC activity, and ebselen oxide hindered c-di GMP binding. Consequently, P. aeruginosa's ability to produce biofilm can be controlled by these two molecules [140].
Many pathogenicity-related bacterial characteristics, including virulence, acid tolerance, biofilm formation, and antibiotic resistance, are thought to be caused by the indole signaling route, which is regarded as one of the most significant signaling pathways [141–144]. According to research by Bunders et al., biofilm formation in S. aureus and E. coli is inhibited by derivatives of desformylflustra bromine (dFBr) through modification of the indole signaling pathway [145].

8. Molecules with unknown mechanism

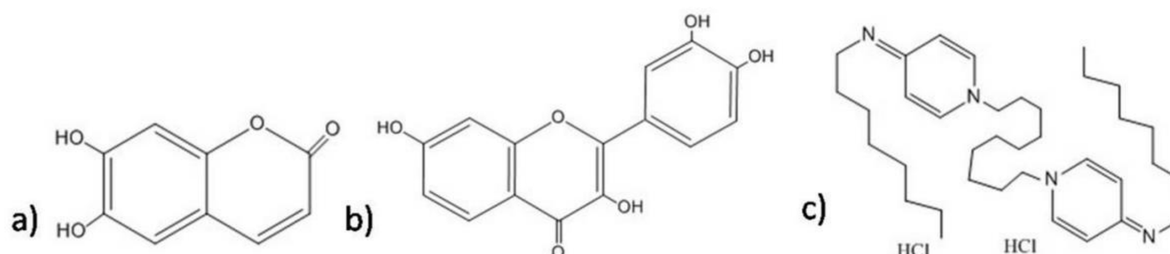

Figure 1: Structures of the antibiofilm molecules with unknown mechanism of action.

a) Esculetin [146], b) Fisetin [147], c) Octenidine hydrochloride [148]
The mechanism of action of several antibiofilm compounds is yet unknown, reports of their effectiveness are rather high. Biofilm inhibition has been seen for the secondary metabolites fisetin and esculetin. Biofilm thickness decreases as a result of esculletin treatment's impact on structural development. Nevertheless, fisetin decreases the thickness of the mature biofilm and also prevents the biofilm from forming, hence decreasing the area covered. Therefore, it is thought that fisetin is a more effective antibiofilm agent than esculetin [149]. While octenidine hydrochloride, a positively charged bispyridinamine, is also proposed as a potent anti-biofilm agent, its exact method of action is yet unknown. Empirical research indicates that this substance has promise as a sanitizer and antibacterial lock solution for both therapeutic and preventive purposes [150].

Conclusions and Future Prospective

Bacterial biofilm production has been extensively researched and understood thus far. A major obstacle in the medical industry is the rise of severe biofilm infections and their resistance to antimicrobial therapy. The major sources of resistant bacteria include fruits, vegetables, dairy products, seafood, poultry products, farm animals, and people. Therefore, it is crucial to look into the best approaches to deal with this issue and identify an antibiotic substitute. Biofilm imaging methods have advanced significantly in response to today's enormous challenge. Three approaches that use super-resolution microscopy include fluorescence photo-activated localization microscopy (FPLAM), photo-activated localization microscopy (PLAM), and stochastic optical reconstruction microscopy (STORM) [151–153]. Compared to CSLM, they generate pictures with far greater resolution by using fluorescent proteins or probes.
Therefore, it is possible to investigate biofilm more often using these approaches. In the search for a meaningful and potent biofilm-busting alternative, several anti-biofilm compounds are being discovered,

and alterations to the various quorum sensing-related signaling pathways are also being considered. Because the cyclic-di GMP (c-di-GMP) signaling pathway is absent in higher eukaryotes, it is a desirable target for the development of naïve anti-biofilm drugs. In addition to this, amyloids' function in bacterial biofilms has gained popularity. By reducing the adhesion of bacterial cells, targeting these amyloids has an impact on the development of biofilms [10]. The current study offers details on the many small compounds with anti-biofilm capabilities and their mechanisms of action.
Each anti-biofilm molecule has a distinct method of action, but a single molecule may have more than one. For instance, EGCG can act by membrane rupture and peptidoglycan breakdown, or it can block the AHL-mediated quorum sensing pathway. A deeper understanding of the nature of biofilms is possible with knowledge of the mechanism of action, which may then be used to the development of novel, effective therapeutic molecules that have the previously identified target of action. The previously known medications' effectiveness may improve as a result. This can be accomplished by appropriate modification or combinatorial treatment, which increases the action of the antibiotics by combining a robust anti-biofilm agent with a previously reported less effective medication against bacterial infections [108].

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