Available online at http://www.ijcrls.com

International Journal of Current Research in Life Sciences
Vol. 12, No. 11, pp. 3492-3501, November, 2023

ISSN: 2319-9490

REVIEW ARTICLE

ANALYTICAL REVIEW OF SELECTED STRATEGIC METHODS IN COMBATING BACTERIAL BIOFILMS IN RECENT TIMES

Loveth O. Fasogbon*[1], Chukwuemeka E Nwankwo[1,2], Adeleke Osho[1] and Samuel N Akpan[3]

[1]Department of Biological Sciences, Redeemer's University, Nigeria
[2]African Centre of Excellence for Genomics of Infectious Diseases (ACEGID), Redeemer's University, Nigeria
[3]African Centre of Excellence for Genomics of Infectious Diseases (ACEGID), Nigeria

Received 20th September, 2023; Accepted 01st October, 2023; Published 30th November, 2023

ABSTRACT

In the production of biofilms by bacteria, there is a continuous aggregation of cells due to complex mechanisms involving inter and intra cellular signalling leading to the creation of the pellicle layer by the proteins and polysaccharides of the external membranes of the cells. These biofilms composed of a rich variety of gram negative and gram-positive bacteria. With the propensity to cause harm and infection, numerous literature have termedbiofilms difficult to control. Hence, biofilms contribute to the global burden of antimicrobial resistance. In this review article, we discuss the current trend in biofilm studies and the methods involved in combating the growth and spread of bacterial biofilms. We discuss contact killing, inhibition of quorum sensing, alterations to the membranes of host cells and peptidoglycan cleavage, inhibition of cell division and dispersion and other methods of evading host defence systems. We discuss control methods like plant extracts and essential oils, non-antibiotic strategies like Antimicrobial photodynamic therapy (APDT), cold atmospheric plasma (CAP), biofomics and other novel, molecular strategies. In conclusion various techniques have been developed to study biofilms and biofilm-embedded microorganisms and their control, with future perspective of the need for continued research to develop more effective and cost-effective methods to prevent and treat biofilm-related infections including nanoparticles.

Key words: *Biofilm production, Antimicrobial resistance, Contact killing, Cell division inhibition, Dispersal inhibition, Antimicrobial photodynamic therapy (APDT), Cold atmospheric plasma (CAP), Biofomics, Nanoparticles and Global burden.*

Citation: Loveth O. Fasogbon, Chukwuemeka E Nwankwo, Adeleke Osho and Samuel N Akpan. 2023. "Analytical review of selected strategic methods in combating bacterial biofilms in recent times" *International Journal of Current Research in Life Sciences*, 12, (11), 3492-3501.

INTRODUCTION

Biofilms are complex microbial communities held together by self-produced polymer matrices, which are made of polysaccharides, secreted proteins, and extracellular DNAs. They offer microbes adaptability, such as increased access to nutritional resources and improved resistance to biocides. The process of biofilm development includes reversible attachment, irreversible attachment, EPS generation, maturation, and dispersal/detachment. Biofilms make pathogens difficult to treat, and they are responsible for two-thirds of all bacterial infections in humans, causing over 60% of microbial illnesses. Biofilms can also spread resistance genes among microorganisms, leading to antimicrobial resistance (AMR), which is currently a serious threat to human and animal health (Kragh *et al*., 2023). The decline in innovative therapeutic compounds is currently plaguing the world, leading to an increasing loss of efficacy of antibiotics, and the transition to a post-antibiotic era. AMR currently causes 700,000 deaths annually and may put 10 million lives at risk by 2050. In 2017, the World Health Organization (WHO) released a comprehensive list of priority pathogens highly resistant to most currently used medicines, including *Acinetobacter baumannii, Staphylococcus aureus, E. coli, Klebsiella spp.,* and *Streptococcus pneumoniae* (Vieira-da-Silva and Castanho, 2022; Michaelis and Grohmann, 2023; Hodin, 2023).

Coresponding author: Loveth O. Fasogbon
Department of Biological Sciences, Redeemer's University, Nigeria

The need to address these issues and the dearth of new antimicrobials provide sufficient incentive to expand the search for viable medications and drug scaffolds from other sources. A considerable portion of newly licensed antibacterials are either natural products directly or are derivatives of natural products, making research efforts centered on natural products an attractive avenue for exploration. In order to find new and beneficial antimicrobial products, researchers need to look into the vast reservoir of compounds found in plants. These compounds contain a variety of known biological activities, including antibacterial capabilities. The World Health Organization also acknowledges the importance of plants as a cornerstone of primary healthcare for more than half of the global population, particularly in countries with limited resources. Plant-based natural products have been acknowledged as a valuable resource that could lead to the development of novel antibacterial compounds with potential novel modes of action. When used as an ingredient in feed and meals, bioactive plant-based products have the ability to improve animals' health and lifestyle (El-Saadony *et al*., 2023).

Biofilm Development: The growth of a biofilm is a dynamic process. When a planktonic bacterium adheres to a surface, it can form a complex biofilm with other organisms. Each organism has a unique way of attaching to surfaces, such as using flagella, pili, proteins, or polysaccharide adhesins. It can be difficult to find and isolate a therapeutic target because microbes can aggregate and form biofilms

on both biotic and abiotic surfaces. The initial adhesion of bacterial cells is the critical stage in biofilm formation. Once attachment has begun, the bacteria have two options, depending on the environment. They can either return to the planktonic phase or continue to develop into a biofilm by adhering to the surface. When an organism enters a biofilm, growth stops and the dispersion phase begins. During this phase, pathogenic cells detach from the biofilm and often cause infections in the host (Qian *et al.,* 2022).

Components of Biofilms: Numerous organisms get together to form multicellular structures called biofilms. The complex of the biofilm is supported by a matrix of secreted polymeric molecules (EPS). This matrix provides nutrition, stability, adhesion, and defense inside the biofilm. Water makes over 91 percent of the biofilm matrix.. Water has two possible uses: it can be a solvent or it can interact directly with microbial cells. Water is a crucial element since it aids in the biofilm's dispersion. The biofilm contains around 5% microorganisms, 2% EPS matrix, and an additional 2% of proteins, DNA, and RNA. Depending on the bacterial makeup and the surrounding conditions, the matrix's structure changes (Subhadra, 2022; Flemming *et al*., 2023).

The Structure and Pathogenesis of Microbial Biofilms: Antimicrobial resistance is brought on by microbial biofilms, which are exopolymeric substances (EPS) that cause microbe attachment to biotic surfaces like host cells or abiotic surfaces like medical equipment. These biofilms also contain molecular components including eDNA and exoenzymes (-lactamase, toxins, etc.), which limits the amount of antibiotic that can diffuse through the biofilm matrix, the amount of persister cells, and the amount of nutrients and oxygen available. Surface proteins and polysaccharide intercellular adhesions (PIA) play crucial roles in the development and production of the biofilm in their pathogenesis (Flemming *et al*., 2023). Infections linked to biofilms include endocarditis, urinary tract infections, septic arthritis, chronic rhinosinusitis, ocular infections, wound infections, and infections linked to medical devices and indwelling devices. Recurrent, incurable infections and medical device failure are the effects of biofilms forming on indwelling medical devices. The detection of microorganism biofilms, their development and dispersion, as well as the antibiofilm and antibacterial activity of drugs against the bacteria in the biofilm, are crucial for treating persistent and recurrent infections. High-throughput screening using a microtiter plate assay technique can be useful for identifying genes involved in the formation of biofilms and measuring genes been expressed as a result of antibiofilm agents and antibacterial action (Di Domenico *et al.,* 2023).

Techniques Used to Study Biofilms and Biofilm-Embedded Microorganisms: Common antimicrobial susceptibility tests, like the broth macrodilution and microdilution techniques, published by the Clinical Laboratory Standards Institute (CLSI), the National Committee for Clinical Laboratory Standards (NCCLS), and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), were never able to provide accurate results in microbes that produce biofilms because they were designed for the detection of the antimicrobials. The National Committee for Clinical Laboratory Standards (NCCLS), the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and the Clinical Laboratory Standards Institute all published standard antimicrobial susceptibility tests, such as the broth macrodilution and microdilution techniques (CLSI). However, because these tests were developed to identify the antimicrobial procedure, they were never reliable for detecting microorganisms that create biofilms. Clinical microbiologists have employed a variety of techniques for the measuring and identification of microbial biofilms in response to agents (Tables 1&2). The modified Robbins device, the Calgary biofilm device, the disk reactor, the Centers for Disease Control (CDC) biofilm reactor, the perfused biofilm fermenter, and the model bladder have all been enhanced as model systems. By supplying details on the mechanisms behind biofilm formation, model systems aid in defining the susceptibility of antimicrobial drugs against microorganisms that create biofilm. For the modified Robbins device, Calgary biofilm device, disk reactor, CDC biofilm reactor, and perfused biofilm fermenter, respectively, the substrates employed are silica disks, plastic pegs, Teflon coupons, plastic needleless connectors, and cellulose acetate filters. Urinary catheters serve as the substrate for the model bladder (UCs). Some biofilm researchers have demonstrated that medical devices with dimensions modified to suitable sizes can also be used as a substratum (abiotic surfaces) for biofilm production by adapting and adjusting to related methodologies. The Calgary biofilm device and modified Robbins device both functions based on viable counts. Before counting, pegs are sonicated in the Calgary biofilm apparatus. Following the sonication, vortexing, and homogenization of the substrates, the methods of disk reactor and CDC biofilm reactor based on direct and viable counts are used. While viable counting is performed in a perfused biofilm fermenter after filters are agitated in sterile distilled water, UCs are directly inspected in a model bladder using scanning electron microscopy (SEM), transmission electron microscopy (TEM), or chemical analysis (Fernández-Barat *et al*., 2023; Kamini *et al*., 2022; Ly *et al*., 2023).

Table 1. The methods used for detection and measurement of biofilms produced on medical devices (Kırmusaoğlu, 2019).

Method	Action of application	Aim
Roll plate	Extraluminal biofilm detection	Growth of biofilm-embedded bacteria
Sonication, vortex, and plate counting	Intraluminal and extraluminal biofilm detection	Growth of biofilm-embedded bacteria
Acridine orange staining	Extraluminal biofilm detection	Direct investigation of biofilm produced on the catheter by microscopy
Streak plating of alginate swab	Investigation of biofilm produced on an indwelling catheter	Growth of biofilm-embedded bacteria

Mechanisms of Antibiotic Resistance in Bacteria Biofilms: An enormous level of antibiotic resistance is displayed by bacteria that are able to grow and survive in biofilms. Because of the stable structural characteristics and close proximity of the bacterial cells within the biofilm, horizontal gene transfer is considerably enhanced, which could lead to the spread of antibiotic-resistant genes among the biofilm's inhabitants. According to the literature that is currently accessible, the following physiological and structural characteristics of biofilm-forming bacteria aid in their gradual development of antibiotic resistance (Michaelis and Grohmann, 2023).

Current strategies to combat bacteria biofilms: Studies have shown several methods which have been proven efficient in the control and eradication of bacteria biofilms and they are described below in Figure 1.

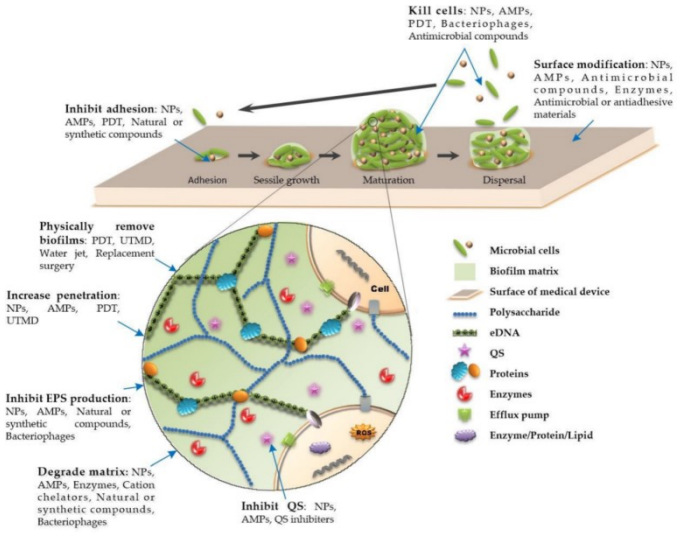

Figure 1. Investigative strategies to eradicate biofilms (Nadar *et al*., 2022)

Antimicrobial Release Agent: To eliminate the microorganisms within the biofilm, antimicrobial chemicals are specifically packed and blended into the dental materials used for various dental treatments. Antibiotics and silver compounds were used in the first antimicrobial treatment for eliminating biofilms. The main benefit of this strategy is that antibacterial substances against biofilms are created even at extremely low concentrations to prevent the growth of bacteria. Additionally, utilizing an antimicrobial releasing agent can result in a high local dose at the location of interest, powerful broad-spectrum antibacterial activity, decreased systemic toxicity, and a lower risk of developing antibiotic resistance. This method does, however, have certain drawbacks, including its limited duration and the possibility of depleting the tooth material's antibacterial reservoir. Various antimicrobial compounds are currently being incorporated into dental materials using nanotechnology, giving them a better antibacterial action and longer lasting (Zhang *et al*., 2022; Hasan and Alhuwaizi, 2022).

Nanoparticles : Removal of oral biofilm using nanoparticles has huge promise. Targeting particular biofilm-forming microorganisms with nanoparticles is possible without altering the normal microflora of the oral cavity because of their excellent antibacterial activity (Namburu *et al*., 2022; Funari and Chen, 2022). The NPs are made up of bioactive NPs, metal NPs, metal-polymer nanocomposites, NPs that release NO/ROS, and NPs that respond to stimuli. NPs are an alternative to conventional antibiotic therapy for illnesses that are linked to biofilms and are multidrug resistant. As antibacterial and antibiofilm agents, several NP types, including organic, inorganic, metal, and green NPs, as well as mixtures of them, have been developed. AgNPs have been employed in a variety of disinfectants because silver (Ag) is an effective antibacterial. AgNPs have a number of antibacterial properties, including the capacity to adhere to and enter microbial cells, increasing membrane permeability and cell disintegration (Logambal *et al*., 2023; El Semary and Bakir, 2022; Madivoli *et al*., 2022). Significant antibiofilm action was discovered in a study using AgNPs against five biofilm-producing, multidrug resistant bacteria (E. coli, A. baumannii, P. mirabilis, K. pneumoniae, and P. aeruginosa). Rifampicin dramatically boosted the retention time and antibacterial activity against S. aureus biofilms when it was encapsulated in poly-L-lysin and added to nanoparticles of poly-lactic acid (PLA) produced using nanoprecipitation. When biologically synthesized AgNPs were used, they inhibited P. aeruginosa and E. coli immature biofilms by 80% and 85%, respectively. Respectively. faecalis AgNPs produced from the medicinal plant Crataeva nurvala dramatically reduced the production of QS-mediated virulence factors such hemolysin, pyocyanin, and protease and prevented P. aeruginosa from forming biofilms (Asma *et al*., 2022). Effective biofilm targeting is made possible by NPs, and work on developing new NPs is ongoing. However, there is still a disconnect between the various formulations being studied in the lab and their practical application in the clinic. Future research and development efforts should focus on increasing NPs' biocompatibility, metabolism, and toxicity as well as their in vivo effectiveness inside the body. Cost-effective large-scale production would also be necessary for the creation of commercial products (Asma *et al*., 2022).

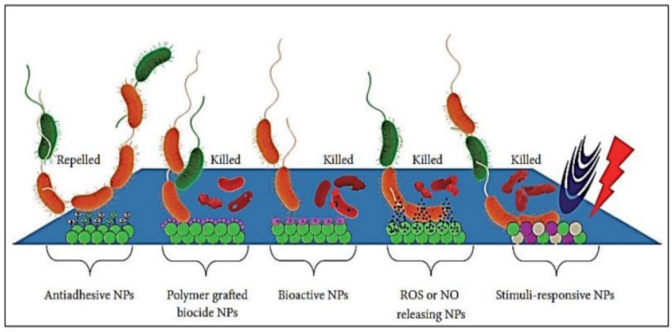

Figure 3. Surface-engineered NPs with various antimicrobial properties' anti-biofilm activity (Asma *et al*., 2022).

Contact-killing: The contact-killing approach is based on the deliberate addition of a variety of antimicrobial agents into dental materials. This method has a number of advantages, such as being non-toxic, having long-lasting antimicrobial action, and being non-irritating. Antimicrobial agents employed in this method range from natural biomolecules like antimicrobial peptides to manufactured chemicals like polycations and quaternary ammonium compounds. QACs have a long history of use as antiseptics and disinfectants for almost 100 years (Osimitz and Droege; 2022). There have reportedly been more than 0.7 million tons of QACs consumed globally to date. In terms of structure, QACs are made up of compounds containing nitrogen , where the N atom is joined by covalent bonds to four distinct groups. The cationic surfactants and antimicrobials known as QACs have a robust and broad-spectrum contact-killing impact against viruses, fungi, malaria, amoebas, and both Gram-positive and Gram-negative bacteria. Although not fully understood, it has been generally accepted that disruption of the bacterial cell membrane structures is the mechanism through which QACs exert their antimicrobial effects (Saverina *et al*., 2023). With pendant QACs, polymeric materials can be synthesized using three different techniques. In the first, polymers with either tertiary ammonium groups or alkyl halides are quaternized. It has been shown that in order to create QA-based dental materials, quaternary ammonium monomers , which can be copolymerized or polymerized to form a polymer network, need be created. Dodecyl pyridinium bromide, which was made commercially available as an antibacterial adhesive system, is the first synthetic QAM to be utilized in antibacterial dental materials (Nadagouda *et al*., 2022).

On a variety of oral bacteria and biofilms, unpolymerized MDPB demonstrates potent antibacterial properties. In previously released papers, other polymerizable QAMs have been created, and their antibacterial properties have been examined using a variety of bacterial strains. High mono-methacrylate concentrations that go beyond the resin polymer network's capacity for polymerization may unavoidably change the structures and mechanical characteristics of those materials. QAMs with dimethacrylate groups have been created to improve their polymerization within a resin network by utilizing the ground-breaking developments in organic chemistry and materials science (Chrószcz-Porębska *et al*., 2023). Two such compounds that have been created are 2-methacryloxylethyl dodecyl methyl ammonium bromide and 2-methacryloxylethyl hexadecyl methyl ammonium bromide (Asma *et al*., 2022; Zhang *et al*., 2020).

Inhibition of AHL-Mediated quorum sensing: Numerous bacteria use N-acyl homo-serine lactones (AHLs) as signaling molecules during quorum sensing to control their population density and to encourage swarming motility. Gram-negative bacteria in particular use AHLs. These signaling molecules are created by a LuxI-type synthase and differ in length and acyl side chain substitutions. These compounds bind to a cognate LuxR-type transcriptional activator protein at specific critical concentrations, which controls the expression of the target gene (Asma *et al*., 2022; Roy *et al*., 2018). Natural furanone produced by the Australian macroalga Dilsea pulchra is converted into a synthetic halogenated furanone molecule as a secondary metabolite product. This substance has the ability to hinder bacterial signaling mechanisms and cell swarm migration. Furthermore, it was proposed that D. pulchra furanones and AHL molecules' structural similarity affects the interactions of putative regulatory proteins with AHL molecules by binding competitively to the receptor (Asma *et al*., 2022; Roy *et al*., 2018). In ecologically relevant concentrations, furanones prevent the surface aggregation characteristics of relevant ecological microorganisms. Furanone 56 inhibits the transcription of the lasB-gfp (ASV) reporter fusion, which is controlled by quorum sensing, by reducing extracellular chitinase and elastase activity while having little to no impact on bacterial growth or protein synthesis. According to studies, furanone penetrates P. aeruginosa's biofilm matrix and targets the rhl system, which is involved in quorum sensing, influencing the expression of genes associated to quorum sensing and biofilm maturity. By changing the biofilm's structure, this molecule speeds up bacterial detachment and causes the bacteria's biomass to be lost from the substrate. Additionally, it was found that furanone facilitates the

expulsion of AHL molecules from Lux R, indicating that furanone is compatible with the corresponding AHL signal for the LuxR receptor site (Liaqat and Farooq, 2023; Vargas *et al*., 2022). The observations regarding furanones are currently supported by a number of experimental findings, including the reduction of virulence factor production and pathogenicity, repression of AHL-dependent bioluminescence expression, and inhibition of quorum sensing-controlled luminescence. Some polyphenols (such as EGCG, tannic acid, and ellagic acid) are thought to follow a similar process to prevent the formation of biofilms, but because they are less effective than furanones, higher concentrations are needed (Bouchelaghem *et al*., 2022; Bae *et al*., 2022). A flavonoid called quercetin affects quorum sensing as well as acting as an anti-biofilm agent against S. aureus. It decreases the adhesion during biofilm formation by inhibiting alginate synthesis in a concentration-dependent manner. Additionally, it causes the induction of swarming movement and lowers exopolysaccharide (EPS) synthesis, which is necessary for the first adhesion of bacteria. Two additional synthetic flavonoids, in addition to quercetin, have been found and are promising antibacterial agents for S. aureus biofilm and scattered cells (Roy *et al*., 2022; Nguyen and Bhattacharya, 2022). According to several additional research, usinic acid inhibits the growth of the S. aureus biofilm and alters the morphology of the P. aeruginosa biofilm. Although the precise mechanism of action is still unknown, researchers have speculated that this might be caused by any quorum sensing interference (Asma *et al*., 2022; Roy *et al*., 2018). A phytochemical called curcumin, which is found in the rhizome of the Curcumalonga plant, has a strong antibiofilm impact by modifying the expression of genes related to quorum sensing and other virulence factors such the synthesis of alginate and swarming motility (Armilda *et al*., 2022).

Membrane Permeabilization or Potential Alteration: Changes in bacterial membrane permeability cause the cytoplasmic membrane to rupture and create pores. Antimicrobial peptides (AMPs) can damage bacterial membranes in one of three ways: I through the barrel-stave pathway caused by pores, (ii) through the toroidal pathway, or (iii) through the carpet (non-pore) mode The lantibiotics are ring-structured peptide antibiotics formed of dehydro-alanine, lanthionine, or thioether amino acids (2-amino isobutyric acids, or lanthionine or methyllanthionine). These synthesized and post-translationally modified peptides disrupt bacterial membranes, preventing them from generating enzymes. The important (pore-forming) lantibiotic subtilin, which is produced by the Gram-positive bacterium B. subtilis, is responsible for causing transmembrane electrostatic potential to dissipate and the release of cytoplasmic solutes from B. subtilis and Staphylococcus simulans membrane vesicles (ATCC 6633 strain) (Yu *et al*., 2022; Zhang and Yang, 2022). The most well-known lantibiotic, nisin, chemically similar to subtilin, prevents the development of the cell wall by forming complexes with lipids I and II. When nisin generates momentary holes, the cytoplasmic membrane may start to leak. Gallidermin and epidermin not only interact with lipid-I, lipid-II, and their intermediates but also participate in the synthesis of lipid-II. By preventing the atl (autolysin) and ica (intercellular adhesin) genes from functioning, gallidermin dramatically reduces the formation of Staphylococcal biofilms. But the antibiofilm effect of gallidermin was greatly hindered by existing biofilms (24 h to 5 days old) (Ning *et al*., 2022; Field *et al*., 2023).Microbial surfactants, also known as biosurfactants (BSs), are amphipathic (and surface-active) molecules produced by microorganisms that have antibacterial properties and hinder bacterial cell adherence to surfaces, disrupting biofilms. Biosurfactants are promising antibiofilm chemicals for biofilm eradication due to their potential antibacterial, anti-adhesive, and dynamically active dispersion capabilities. When combined with caprylic acid, sophorolipid BSs promote membrane permeability and prevent B. subtilis, E. coli, and P. aeruginosa from developing biofilms. S. aureus's ability to generate biofilms was reduced by BSs produced by Lactobacillus casei (Sondhi, 2023; Mgomi *et al*., 2023).

Peptidoglycan Cleavage: In addition to altering the protein composition, the quantity of teichoic acid in the bacterial cell wall, and the potential release of signaling molecules that could affect the expression of the biofilm gene, the peptidoglycan cleavage prevents the formation of biofilms in a number of other ways as well. The cell walls of many bacteria have a layer of peptidoglycan, which is composed of amino acids and carbohydrates. Endolysins are a distinctive class of peptidoglycan hydrolases encoded by bacteriophages. They attach to the bacterial cell wall and break it down; this causes hypotonic cell lysis, bacterial cell death, and the release of offspring bacteriophages. They are frequently species-specific. Endolysin can break up in vitro biofilms and be effective against multidrug-resistant bacteria, such as PlyC (a particular Streptococcal bacteriophage (Johnston *et al*., 2022; Liu *et al.,* 2023). Epigallocatechin gallate, a polyphenol molecule, interacts with peptidoglycan and damages bacterial cell walls, which inhibits the growth of bacteria and ultimately prevents the primary or docking stage of biofilm development. Tannic acid, a polyphenolic molecule, can prevent S. aureus from forming biofilms without affecting bacterial growth. Tannic acid's method of action is dependent on the immune-dominant Staphylococcal Antigen-A, IsaA, a hypothesized lytic transglycosylase that breaks down peptidoglycans. Transglycosylase, an enzyme that mimics a lysozyme, causes the b-1,4 glycosidic bond cleavage between N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM). Tannic acid can also stop biofilm development by boosting IsaA extracellular levels (Acet *et al*., 2023; Jailani *et al*., 2022).

Inhibition of Bacterial Cell Division: The development of bacterial biofilms depends on the bacteria's ability to divide their cells. Cytoplasmic proteins promote cellular viability and aid in cell division. Only a few number of peptides with antimicrobial characteristics are able to penetrate the bacterial cytosol through the flip-flop of phospholipids or by opening channels in the membrane to prevent the activity of cytoplasmic proteins.Drosocin, pyrrhocoricin, and apidaecin are a few proline-rich antimicrobial peptides (AMPs) that have the capacity to connect with a heat shock protein of bacteria (DnaK), preventing the start of cDNA (chromosomal DNA) replication. The bacteria may also be killed by them by severing the links between DnaJ (the heat shock protein) and DnaK. Bacterial cells can be invaded by proline-rich AMPs, which connect to the ribosome tunnel and hinder bacteria from making proteins (Phuket *et al*., 2023; Erdem Büyükkiraz and Kesmen, 2022).

Inhibition of Biofilm Dispersion: Biofilm breakdown is based on a set of processes that affect cellular physiology and deteriorate the extracellular matrix. The majority of bacterial species have the ability to create extracellular enzymes or surfactants that can break down or dissolve the biofilm matrix. Bacterial cells are detached from the biofilm and discharged into the environment if the matrix is removed. DNases, proteases, and surfactants are a few substances that can facilitate the active dispersion of biofilms (Pakkulnan *et al*., 2023; Ramírez-Larrota and Eckhard, 2022). The breakdown of biofilms has also been connected to the production of extracellular proteases. When biofilms are disassembled, nuclease, also known as effective DNase, micrococcal nuclease, and thermonuclease, acts as an internal mediator to make it easier for bacteria to separate from biofilms. Restrictions enzymes and DNases have been associated to biofilm dispersal. D-tyrosine can stop bacterial cells from adhering, hence preventing the development of biofilms. Additionally, it can start the disintegration of P. aeruginosa and B. subtilis biofilms at low concentrations. In both Gram-positive and -negative bacteria, the D-tyrosine influence on extracellular protein and EPS synthesis is dose-dependent (Su *et al*., 2023; Asare *et al*., 2022). According to studies, D-tryptophan, D-histidine, and D-cysteine can inhibit the growth of A. baumannii biofilms by up to 35-86% at a concentration of 2 mM. Additionally, at a dosage of 4 mM, D-tyrosine, D-cysteine, and D-tryptophan reduced the development of P. aeruginosa biofilms by up to 10–30%. Bhoopalan *et al*. recently proposed using the nagZ protein, which is involved in recycling peptidoglycan, in breaking established Neisseria gonorrhoeae biofilms, but the exact mechanism of action is still unclear (Asma *et al*., 2022; Cen C *et al*., 2022).

Biofilm Inhibition via Polysaccharides: It is well recognized that extracellular polysaccharides (EPSs) are crucial components of many biofilms. It has been demonstrated that EPSs can disrupt the biofilm

matrix already in place in addition to preventing the growth of new biofilms. An EPS with the name EPS-273 that was isolated from the marine bacteria P. stutzeri 273 prevents the growth of P. aeruginosa's biofilm by specifically inhibiting the production of several virulence factors such rhamnose, exo-protease, and pyocyanin. Pyocyanin production is decreased by EPS-273, which has a minimal impact on H2O2 production. Additionally, it has the capacity to prevent the release of eDNA, which has been shown to be crucial for the development of stable biofilms (Champion *et al*., 2022; Sudhakaran *et al*., 2022). It has been demonstrated that the antioxidant EPS-273 reduces diseases linked to biofilms. P. aeruginosa can be used in healthcare settings to lower nosocomial infections and in the food industry to prevent food spoilage because it is resistant to EPS-273. Exopolysaccharide A101, which comes from Vibrio cholerae QY101, has the ability to break up P. aeruginosa biofilms. Polysaccharides from animals, plants, and algae have also been shown to serve as antibiofilm molecules in addition to those of bacterial origin. (Champion *et al*., 2022; Trilokesh *et al*., 2023).

Cyclic di-GMP System Signaling Inhibition: The three main types of bacterial communities are I planktonic (which can cause acute bacterial infections but is usually quickly treated with antibiotics), (ii) biofilm (which is generally difficult to treat with antibiotics), and (iii) dispersed (which is defined as a transition between planktonic and biofilm states) biofilms. Bacterial biofilms can spread among and within different hosts thanks to the dispersal process. Cyclic di-GMP (c-di-GMP), a secondary messenger molecule, plays a role in the development of bacterial biofilms, and the signaling system that controls c-di-GMP can be modified to affect bacterial biofilm growth. Microbiological cells limit the amount of c-di-GMP when under stress, such as in nitrosative and famine circumstances, by activating phosphodiesterase, which causes biofilms to disperse (Shimizu *et al*., 2022; Lichtenberg *et al*., 2022). The inclusion of an iron-chelating agent along with an antibacterial and dispersion agent has also been shown to potentially remove several biofilms. A. baumannii and P. aeruginosa biofilm development is inhibited by a number of small molecules, such as LP-1062, LP-3134, LP-3145, and LP-4010. These compounds do this by inhibiting diguanylate cyclase (DGC), which prevents the generation of c-di-GMP. Only two of the aforementioned small compounds were not hazardous to eukaryotic cells at the dosages utilized, despite the fact that all of them have been shown to be effective P. aeruginosa biofilm dispersal inhibitors (Liaqat and Farooq, 2023; Repac Antić *et al*., 2022).

Use of Natural Products

-Honey: The most popular natural product with therapeutic, antibacterial, anti-inflammatory, antioxidant, and wound-healing characteristics is probably honey. There have been reports of significant antibacterial activity against 60 different bacterial and fungus species. Honey has been found to be a powerful inhibitor of the growth of Streptococcus pyogenes biofilms. Honey may be used as a treatment for enterococcal infections connected to biofilms because it was found to be effective against the development of enterococcal biofilms (You *et al*., 2022; Jiang *et al*., 2022). It has been demonstrated that honey, even in little dosages, can greatly reduce the production of Enterohaemorrhagic E. coli (O157:H7) biofilms by inhibiting QS and bacterial virulence genes without harming cell growth. Honey's strong antibacterial properties allow it to stop bacterial adhesion and biofilm formation at high concentrations. Honey has antibacterial effects, but because bee defensin-1, another antimicrobial peptide, inhibits microbial viability, it can also prevent the growth of biofilms (Das *et al*., 2022; Deglovic *et al*., 2022).

-Plant Extracts: The primary mechanisms by which natural chemicals impair QS and biofilm development are through the suppression of cellular adhesion, blockage of polymer matrix synthesis, and reduction of virulence factor production. Plant extracts and their active components have been researched for their ability to eliminate microbial biofilms (Chakrabarty *et al*., 2022). The growth and maintenance of plant cells depend on primary metabolites. Among them are vitamins, amino acids, lipids, carbohydrates, proteins, nucleotides, and other compounds. Secondary metabolites include things like alkaloids, steroids, flavonoids, tannins, terpenoids, saponin, and other compounds. They are secondary metabolites with beneficial biological properties like anti-inflammatory, antimicrobial, anti-bacterial, and antifungal action., etc (Chakrabarty *et al*., 2022; Alavi *et al*., 2022). Numerous plant extracts, including those from Rhodiola crenulata, Epimedium brevicornum, Dolichos lablab, Polygonum cuspidatum, Malus pumila, Bridelia micrantha, Moringa olifera, Talinum triangulare, Vernonia amygdalina, Annona muricata, and Anarcardium occidentale, have shown evidence of anti-biofilm activity. Extracts of E. brevicornum and P. cuspidatum, as well as their most potent components, resveratrol and icariin, demonstrated strong anti-biofilm activity even when administered at dosages below their MICs (Ce *et al*., 2020). In many parts of the world, plants are trusted sources for the treatment of ailments. They are gifts from nature that have been used for countless diseases since the dawn of time. Since 1977, WHO has acknowledged this practice in its policy document (Selvi *et al*., 2022, El-Saadony*et al*., 2023). Any plant that has substances that can be used therapeutically or that act as building blocks for the creation of powerful pharmaceuticals in one or more of its organs is considered to be a medicinal plant, according to WHO (1991). Fundamentally, plant cells are chemical factories that house a wealth of therapeutically valuable phytocompounds with the potential to be transformed into powerful antimicrobial substances. Phytochemicals are naturally occurring, bioactive chemicals that give plants their therapeutic properties. Primary metabolites and secondary metabolites are the two main categories of phytochemicals (El-Saadony *et al*., 2023). Over 30% of all plant species are now used medicinally, although the WHO estimates that about 21,000 plant species have medicinal potential. Different portions of the many plant species, such as the leaves, stems, bark, roots, etc., have various chemical components that make them effective against illnesses. These plants include and others (Ghosh *et al*., 2022).

-Essential Oils: Essential oils (EOs) are volatile compounds that are collected from organic plants and have been widely used to combat a number of infections. As a result of their antibacterial and preservation qualities, EOs are widely utilized in the food business. The cell walls of germs are selectively damaged by EOs, and it has been shown that distinct EOs inactivate microbes without causing the development of antibiotic resistance. Surprisingly, because to their quick and simple breakdown, low toxicity, and accessibility to a wide variety of EOs, they are potent natural anti-biofilm agents. Cinnamon oil, which is often applied in the food industry, has been effective in preventing the biofilm formation of Lactobacillus plantarum, S. mutans, and S. epidermidis (Esposito and Turku, 2023). Another well-known EO made from Cuminum cyminum is cumin oil. It is a member of the Apiaceae family of fragrant medicinal plants, which is utilized extensively in both the food and drug industries. Disorders of the digestive system (as a eupeptic and carminative), cough and bronchopulmonary disorders (as an astringent), etc. have all been treated with cumin oil (Ghannay *et al*., 2022). Safoura *et al*. investigated the efficiency of cumin seed oil against K. pneumoniae biofilms and found that ciprofloxacin combined with cumin oil increased efficacy and decreased biofilm development. At low concentrations, oregano oil prevented S. aureus, S. sciuri, S. haemolyticus, and S. lugdunensis from forming biofilms. Tea tree essential oil (TTO) possesses potent antibacterial properties, but when combined with regular ciprofloxacin, the action against P. aeruginosa biofilm formation was markedly increased. The results revealed that CIP and TTO combined to effectively reduce P. aeruginosa biofilm biomass by 70%. Szczepanski and Lipski provided evidence of the effectiveness of cinnamon, oregano, and thymol essential oils against three biofilm-forming bacterial strains (Stenotrophomonas, Acinetobacter, and Sphingomonas). Two of these three essential oils were found to have inhibitory effects on biofilm development at the minimal inhibitory concentration (Roy *et al*., 2018).

Non-Antibiotic Strategies

-Antimicrobial photodynamic therapy (APDT): Acridine hydrochloride and visible light were employed to inactivate *Paramecia caudatum*, which gave rise to the APDT technique. The

use of APDT for the treatment of dental caries, periodontal, and endodontic diseases has increased recently due to its disinfecting effect on a variety of oral microbial pathogens and biofilms. A photosensitizer interacts with low-energy laser light in the presence of oxygen to produce reactive oxygen species, which is the basis for APDT's operation (ROS). The oxidative harm to the bacterial DNA and cell membrane system is what is thought to be responsible for APDT's bactericidal effects. Photosensitizers may be given in a variety of methods depending on the type of agents, including oral consumption, topical application, or intravenous injection (Zhao *et al*., 2022; Dantas *et al*., 2022). Due to the simple molecular structure of ROS, microbes will acquire antimicrobial resistance to APDT. The quick eradication of the offending oral bacteria, the absence of systemic disturbance, and the avoidance of adverse effects on healthy oral cells and tissues are additional benefits of APDT (Rath *et al*., 2021). Dental caries is a disorder in which teeth decay as a result of acids produced by oral bacterial biofilms (Álvarez*et al*., 2022). The APDT technique can be used to prevent dental caries by encouraging the dispersion of produced biofilms and eliminating microorganisms inside carious lesions.. The use of APDT is thought to have a number of benefits, including minimal antibiotic resistance, non-invasiveness to non-cancerous lesions, and quick cariogenic bacterial death. Increasing experimental evidence has shown that cariogenic bacteria, whether they are planktonic or in biofilm form, are vulnerable to APDT. For instance, it has been demonstrated that the photosensitizer toluidine blue O induced APDT is effective against S. mutans teeth decaying (Afrasiabi *et al*., 2022; Pourhajibagher*et al*., 2022; Li *et al*., 2022).

Recently, root surface caries were stopped in vivo and in vitro by combining APDT with others treatments like the casein phosphopeptide-amorphous calcium phosphate or the dental plaque-disclosing drug erythrosine. There have, however, also been reports of negative results. The use of an APDT approach using methylene blue as the photosensitizer exhibited low efficacy on getting rid of cariogenic bacteria in an in vitro multi-species biofilm model. As a result, additional research is needed to confirm the antimicrobial efficiency of APDT and to improve its treatment parameters, particularly randomized clinical trials (RCTs) (Sabino *et al*., 2023). Recently, APDT has also been used to both in vitro and in vivo target bacteria in root canal systems. When receiving APDT treatment in the root canal system, the photosensitizers may enter the periapical tissues through the root apex. After a photosensitizer is activated by light, this iatrogenic effect may negatively impact the condition of periapical host cells. To guarantee that germs are removed and host cells are preserved, it is crucial to identify the therapeutic window. The safety of APDT has been studied in several in vitro experiments up to this point. Methylene blue, a commonly used photosensitizer, killed fibroblasts and E. coli cells up to 36% and 100% in concentrations ranging from 10 mol L-1 to 100 mol L-1. Respectively, following exposure to red light. As an adjuvant to conventional endodontic therapy, APDT has more recently been employed in a clinical setting for root canal disinfection. Other dental uses for APDT include cleaning acrylic denture surfaces, treating oral lichen planus and periimplantitis, and treating periimplantitis (Ensafi *et al*., 2022; Jiao *et al*., 2019).

Despite encouraging results, there are still a few considerations that must be made to ensure successful therapy outcomes. Some of these factors include the kind of photosensitizer used, the right tissue penetration, especially for deep injuries, and the combination of several therapeutic modalities with APDT. Recently, there has been a lot of interest in biodegradable polymeric materials and NPs containing photosensitizer agents. These materials have a number of advantages, including: (1) better biocompatibility and biodegradability; (2) decreased photosensitizer aggregation; and (3) increased bactericidal efficiency due to increased binding between photosensitizers and bacteria. With little adverse effects noted, APDT successfully eliminated dangerous microorganisms. The main adverse effect that has been noted is the persistence of residual skin photosensitivity caused by photosensitizer accumulation. This persistence can range from a few days to a few weeks, depending on the photosensitizer that was used. Therefore, patients should be advised to refrain from exposing their skin and eyes to bright light or sunshine until the photosensitizer has been entirely removed. Additionally, APDT has not been approved for use in dental applications by the American Food and Drug Administration. Clinical research should receive local institutional review board permission before beginning treatment. The development of globally relevant recommendations with consistent parameters is necessary to direct clinical applications in this area (Fabio *et al*., 2023; Gnanasekar *et al*., 2023).

-Cold atmospheric plasma (CAP): CAP is a practical non-antibiotic option for the treatment and management of biofilm infections (Fallon *et al*., 2022; Kim *et al*., 2022). The CAP method makes use of a highly reactive combination of ions, electrons, radical species, molecules in the ground or excited state, and electromagnetic radiation quanta (UV photons and visible light).Unlike typical plasma technology, CAP is operated in an atmosphere, making it possible for in vivo applications without endangering the nearby tissues. A growing body of experimental data has shown that CAP is effective at getting rid of a wide range of bacteria, including Gram-negative P. aeruginosa and E. coli. Gram-positive E. coli, S. aureus B. subtilis and multidrug resistant species like M. luteus, which is resistant to methicillin. Fungi spp. and bacterial biofilms, as well as for regulating Candida albicans (Csadek *et al*., 2023; Dikyol and Ercan, 2022; Das *et al*., 2023). For instance, CAP dramatically lowered the bacterial burden in chronic wounds and effectively reduced 94% of the normal bacterial skin flora. Recent research has shown that CAP can reduce and inactivate harmful human viruses using bacteriophage models. Reactive oxygen and nitrogen species are currently the most generally acknowledged CAP action mechanisms (RONS). The majority of bacteria are vulnerable to RONS, which result in oxidative damage to their DNA, proteinaceous enzymes, and cell membrane. It has also been discussed how charged particles, electrons, and ions work to inactivate microorganisms. The mechanical rupture of the bacterial cell membrane results from charge accumulation on the membrane. Recent dentistry applications for the CAP technique include root canal therapy and dental implant surface modification. It has long been understood that microbial infection is the main cause of pulpitis and apical periodontitis (Cortázar *et al*., 2022; Soheili *et al*., 2022; Haghighi *et al*., 2023).

The CAP treatment successfully killed several oral cariogenic bacteria that are responsible for the development of secondary caries and disturbance of the stability of resin-dentin connections using a variety of tooth-imimicking substrates, cavity models, dentin, and enamel. It is possible to get rid of oral single-species biofilms of A. naeslundii, C. albicans, S. gordonii, S. mutans, S. oralis, and S. sanguinis by using CAP twice daily for 10 to 30 seconds each. Nevertheless, a large number of research covered the shortcomings of CAP, specifically the ineffective clearance of biofilms. By preventing CAP from penetrating the deep layer and exerting its bactericidal effect on the bacteria at the biofilms' base, the polymeric matrix in biofilms restricts CAP's effectiveness. A significant effect is the Biofilm layer's thickness. Furthermore, the susceptibility of microbial pathogens and biofilms to CAP varies with species, strains of the same species, and the composition of reactive species produced by various CAP devices. However, it has been demonstrated that CAP treatment has no negative effects on the mucosa and periodontal tissues, allaying worries about CAP's potentially negative effects on health when used for orally (Jiao *et al*., 2019; Jungbauer *et al*., 2022; Sun *et al*., 2022).
The plasma-derived RONS permeate the biofilm and inflict oxidative harm on its DNA and proteinaceous enzymes, leading to cell death and rupture of the cell membrane (Jiao *et al*., 2019).

Biofomics: A cutting-edge platform called biofomics allows for the systematic and extensive collection, processing, and analysis of data from high-throughput research. To better understand the mechanisms behind biofilm development, the Biofilms Structural Database (BSD) compiles structural, mutagenesis, kinetics, and inhibitory data. It contains curated data on 425 protein and enzyme structures that are important for the growth and development of 42 different bacteria's

biofilms. The knowledge of these structures, along with the enormous body of work dispersed throughout the scientific literature, offers new opportunities to understand biofilms at the structural and atomic level. These data include amino acid sequences, kinetic and mutagenesis data, and inhibitory activity of known molecules. The most recent breakthrough has allowed for a change in study emphasis from the cellular to the molecular level, opening up a whole new field for the creation of antibiofilm therapies. Particularly, the newly accessible structures present an alluring alternative for the rational design of novel medications and for the use of methodologies like docking, virtual screening, quantitative structure-activity relationship (QSAR) models, molecular dynamics, and others. The BSD was developed to organize all of the structural data that is now available on these fascinating topics, and it provides current atomic information on the proteins and enzymes involved in biofilm creation and development (Magalhães *et al*., 2020).

Data Selection: The BSD primarily concentrates at the molecular level. But biofilm research incorporates a range of scales. In order to provide a comprehensive repository of structural data on biofilm research that could be combined with data from other databases, beyond the molecular level, to link other fields, the database was developed (Cesara *et al*., 2022; Sleutel *et al*., 2023). A thorough literature search is done in order to find out the necessary structural information on the proteins and enzymes involved in the creation of biofilms. The ISI Web of Science, Scopus, and PubMed databases were searched using combinations of biofilm-related keywords, such as "biofilm," "quorum sensing," "motility," "dispersion," and other important phrases, with the keywords "structure," "X-ray," "NMR," and "cryo-EM" (cryogenic electron microscopy). Additionally, searches on the Protein Data Bank (PDB) of the Research Collaboratory for Structural Bioinformatics (RCSB) were made. Cross-referencing was done on many databases, including ChEMBL, BindingDB, ExPASy, KEGG, and UniProt, to provide further details for each protein. Direct links to other drug-like compounds that have been tried against that biofilm protein are included in the BSD, together with details on the kinetics and binding affinities of mutant variations and genomic and proteomic information pertaining to each individual protein (Magalhães *et al*., 2020). As research into biofilms advances and new structures are made available on the PDB database, the BSD will continue to be created, updated, and maintained to ensure that it continues to be a valuable and accurate tool for researching biofilms from a molecular perspective.

CONCLUSION

Antimicrobial resistance is a complex issue caused by several physiological and structural characteristics of biofilm-forming bacteria, which exhibit a high level of antibiotic resistance. Various techniques have been developed to study biofilms and biofilm-embedded microorganisms, including viable counting, scanning electron microscopy, transmission electron microscopy, and chemical analysis. The use of nanoparticles, contact-killing agents, natural compounds, antimicrobial peptides, antibiotics, and biosurfactants are promising strategies for preventing and disrupting biofilms, hindering bacterial cell adherence, preventing biofilm formation, and inhibiting biofilm dispersion. Future prospective: There is a need for continued research to develop more effective and cost-effective methods to prevent and treat biofilm-related infections. Nanoparticles, particularly AgNPs, hold promise as an alternative to conventional antibiotic therapy for multidrug-resistant illnesses. However, more work is needed to increase their biocompatibility, toxicity, and in vivo effectiveness for clinical application. The use of natural compounds, including furanones, polyphenols, flavonoids, usinic acid, and curcumin, should also be explored further as potential antibacterial agents against biofilm-related infections. Additionally, biosurfactants and peptidoglycan cleavage hold potential to prevent bacterial cell adherence and biofilm formation. Further research should also focus on the development of new antimicrobial agents and strategies to disrupt biofilms, including the inhibition of bacterial cell division and biofilm dispersion through extracellular enzymes or surfactants.

REFERENCES

Acet, Ö., Dikici, E., Acet, B. Ö., Odabaşı, M., Mijakovic, I., & Pandit, S. 2023. Inhibition of bacterial adhesion by epigallocatechin gallate attached polymeric membranes. *Colloids and Surfaces B: Biointerfaces,* 221, 113024.

Afrasiabi, S., Partoazar, A., Chiniforush, N., & Goudarzi, R. 2022. The potential application of natural photosensitizers used in antimicrobial photodynamic therapy against oral infections. *Pharmaceuticals,* 15(6), 767.

Alavi, M., Hamblin, M. R., & Kennedy, J. F. 2022. Antimicrobial applications of lichens: Secondary metabolites and green synthesis of silver nanoparticles: A review. *Nano Micro Biosystems*, 1(1), 15-21.

Álvarez, S., Leiva-Sabadini, C., Schuh, C. M., & Aguayo, S. 2022. Bacterial adhesion to collagens: implications for biofilm formation and disease progression in the oral cavity. *Critical Reviews in Microbiology,* 48(1), 83-95.

Armilda, L. H. V., Puspitasari, A., Indriyana, M., Nuraeni, T., & Aji, N. 2022, September. ANTIBIOFILM DETERMINATION OF Curcuma longa L. RHIZOME AS DRUG CANDIDATES IN HANDLING Propionibacterium acnes RESISTANCE. *In International Conference on Medical Laboratory Technology* (Vol. 2, No. 1, pp. 92-105).

Asare, E. O., Mun, E. A., Marsili, E., & Paunov, V. N. 2022. Nanotechnologies for control of pathogenic microbial biofilms. *Journal of Materials Chemistry B,* 10(27), 5129-5153.

Asma, S. T., Imre, K., Morar, A., Herman, V., Acaroz, U., Mukhtar, H., Arslan-Acaroz, D., Shah, S. R. A., & Gerlach, R. 2022. An Overview of Biofilm Formation–Combating Strategies and Mechanisms of Action of Antibiofilm Agents. Life, 12(8)

Bae, J. Y., Seo, Y. H., & Oh, S. W. 2022. Antibacterial activities of polyphenols against foodborne pathogens and their application as antibacterial agents. *Food Science and Biotechnology*, 31(8), 985-997.

Bouchelaghem, S., Das, S., Naorem, R. S., Czuni, L., Papp, G., & Kocsis, M. 2022. Evaluation of total phenolic and flavonoid contents, antibacterial and antibiofilm activities of Hungarian Propolis ethanolic extract against Staphylococcus aureus. *Molecules,* 27(2), 574.

Ce, A., Cjo, A., Nl, U., Fm, C., & Il, A. 2020a. Phytochemical and Antimicrobial analysis of leaves of Bridelia micrantha, Cassytha filiformis, Euphorbia hirta and Securinega virosa. ~ 581 ~ *Journal of Pharmacognosy and Phytochemistry,* 9(3)

Cen, C., Chen, J., Wu, X., Fu, L., & Wang, Y. 2022. Screening inhibitor to prevent the psychrotrophic growth of Pseudomonas fluorescens by using molecular simulation. *Food Control,* 134, 108557.

Cesaria, M., Alfinito, E., Arima, V., Bianco, M., & Cataldo, R. 2022. MEED: A novel robust contrast enhancement procedure yielding highly-convergent thresholding of biofilm images. *Computers in Biology and Medicine,* 151, 106217.

Chakrabarty, S., Mishra, M. P., & Bhattacharyay, D. 2022. Targeting Microbial Bio-film: an Update on MDR Gram-Negative Bio-film Producers Causing Catheter-Associated Urinary Tract Infections. *Applied Biochemistry and Biotechnology,* 194(6), 2796-2830.

Champion, M., Portier, E., Vallée-Réhel, K., Linossier, I., Balnois, E., Vignaud, G., ... & Faÿ, F. 2022. Anti-Biofilm Activity of a Hyaluronan-like Exopolysaccharide from the Marine Vibrio MO245 against Pathogenic Bacteria. *Marine Drugs,* 20(11), 728.

Chrószcz-Porębska, M. W., Barszczewska-Rybarek, I. M., Kazek-Kęsik, A., & Ślęzak-Prochazka, I. 2023. Cytotoxicity and Microbiological Properties of Copolymers Comprising Quaternary Ammonium Urethane-Dimethacrylates with Bisphenol A Glycerolate Dimethacrylate and Triethylene Glycol Dimethacrylate. Materials, 16(10), 3855.

Cortázar, O. D., Megía-Macías, A., Moreno, S., Brun, A., & Gómez-Casado, E. 2022. Vulnerability of SARS-CoV-2 and PR8 H1N1 virus to cold atmospheric plasma activated media. *Scientific Reports,* 12(1), 263.

Csadek, I., Vankat, U., Schrei, J., Graf, M., Bauer, S., Pilz, B., ... & Paulsen, P. 2023. Treatment of ready-to-eat cooked meat products with cold atmospheric plasma to inactivate Listeria and Escherichia coli. Foods, 12(4), 685.

Dantas, K. C. F., Rosário, J. D. S., & Silva-Caldeira, P. P. 2022. Polymeric Nanosystems Applied for Metal-Based Drugs and Photosensitizers Delivery: The State of the Art and Recent Advancements. *Pharmaceutics,* 14(7), 1506.

Das, N., Ragavan, M. L., & Mandal, S. K. 2022. Probiotics in the prevention and treatment of infections with Helicobacter pylori, Enterohemorrhagic Escherichia coli, and Rotavirus. In Probiotics in the Prevention and Management of Human Diseases (pp. 281-304). Academic Press.

Das, S., Prakash, G. V., Mohapatra, S., Kar, S., Bhatt, S., Gautam, H., ... & Mallick, S. R. 2023. Antimicrobial efficacy of argon cold atmospheric pressure plasma jet on clinical isolates of multidrug-resistant ESKAPE bacteria. *IEEE Transactions on Radiation and Plasma Medical Sciences.*

Deglovic, J., Majtanova, N., & Majtan, J. 2022. Antibacterial and Antibiofilm Effect of Honey in the Prevention of Dental Caries: A Recent Perspective. *Foods,* 11(17), 2670.

Di Domenico, E. G., Oliva, A., & Guembe, M. 2022. The current knowledge on the pathogenesis of tissue and medical device-related biofilm infections. *Microorganisms*, 10(7), 1259.

Dikyol, C., & Ercan, U. K. 2022. Evaluation of Penetration Depth of Antimicrobial Effect by Cold Atmospheric Plasma Treatment In Vitro. *Plasma Medicine*, 12(1).

El Semary, N. A., & Bakir, E. M. 2022. Multidrug-resistant bacterial pathogens and public health: the antimicrobial effect of cyanobacterial-biosynthesized silver nanoparticles. *Antibiotics,* 11(8), 1003.

El-Saadony, M. T., Zabermawi, N. M., Zabermawi, N. M., Burollus, M. A., Shafi, M. E., Alagawany, M., ... & Abd El-Hack, M. E. 2023. Nutritional aspects and health benefits of bioactive plant compounds against infectious diseases: a review. *Food Reviews International,* 39(4), 2138-2160.

El-Saadony, M. T., Zabermawi, N. M., Zabermawi, N. M., Burollus, M. A., Shafi, M. E., Alagawany, M., ... & Abd El-Hack, M. E. 2023. Nutritional aspects and health benefits of bioactive plant compounds against infectious diseases: a review. *Food Reviews International,* 39(4), 2138-2160.

Ensafi, F., Fazlyab, M., Chiniforush, N., & Akhavan, H. 2022. Comparative effects of SWEEPS technique and antimicrobial photodynamic therapy by using curcumin and nano-curcumin on Enterococcus faecalis biofilm in root canal treatment. *Photodiagnosis and Photodynamic Therapy*, 40, 103130.

Erdem Büyükkiraz, M., & Kesmen, Z. 2022. Recombinant expression and coexpression of oyster defensin and proline-rich peptide in Komagataella phaffii. *Biotechnology and Applied Biochemistry,* 69(5), 1998-2007.

Esposito, M. M., & Turku, S. 2023. The Use of Natural Methods to Control Foodborne Biofilms. *Pathogens,* 12(1), 45.

Fabio, G. B., Martin, B. A., Dalmolin, L. F., & Lopez, R. F. V. 2023. Antimicrobial photodynamic therapy and the advances impacted by the association with nanoparticles. *Journal of Drug Delivery Science and Technology,* 104147.

Fallon, M., Conway, J., Kennedy, S., Kumar, S., Daniels, S., & Humphreys, H. 2022. The Effect of Cold Plasma Operating Parameters on the Production of Reactive Oxygen and Nitrogen Species and the Resulting Antibacterial and Antibiofilm Efficiency. *Plasma Medicine,* 12(1).

Fernández-Barat, L., Vázquez Burgos, N., Alcaraz, V., Bueno-Freire, L., López-Aladid, R., Cabrera, R., ... & Torres, A. 2023. The value of biofilm testing to guide antimicrobial stewardship in chronic respiratory diseases. *Frontiers in Cellular and Infection Microbiology,* 13, 446.

Field, D., Fernandez de Ullivarri, M., Ross, R. P., & Hill, C. 2023. After a century of nisin research, where are we now and where are we going?. *FEMS Microbiology Reviews,* fuad023.

Flemming, H. C., van Hullebusch, E. D., Neu, T. R., Nielsen, P. H., Seviour, T., Stoodley, P., ... & Wuertz, S. 2023. The biofilm matrix: Multitasking in a shared space. *Nature Reviews Microbiology,* 21(2), 70-86.

Funari, R., & Shen, A. Q. 2022. Detection and characterization of bacterial biofilms and biofilm-based sensors. *ACS sensors,* 7(2), 347-357.

Ghannay, S., Aouadi, K., Kadri, A., & Snoussi, M. 2022. In Vitro and In Silico Screening of Anti-Vibrio spp., Antibiofilm, Antioxidant and Anti-Quorum Sensing Activities of Cuminum cyminum L. Volatile Oil. Plants, 11(17), 2236.

Ghosh, S., Sarkar, T., & Chakraborty, R. 2022. Ankol plant (Alangium salvifolium)-The treasure trove of bioactives and medicinal potential. *Food Bioscience,* 102230.

Gnanasekar, S., Kasi, G., He, X., Zhang, K., Xu, L., & Kang, E. T. 2023. Recent advances in engineered polymeric materials for efficient photodynamic inactivation of bacterial pathogens. *Bioactive Materials,* 21, 157-174.

Haghighi, L., Azizi, A., Vatanpour, M., & Ramezani, G. 2023. Antibacterial Efficacy of Cold Atmospheric Plasma, Photodynamic Therapy with Two Photosensitizers, and Diode Laser on Primary Mandibular Second Molar Root Canals Infected with Enterococcus faecalis: An In Vitro Study. *International Journal of Dentistry,* 2023.

Hasan, S. M., & Alhuwaizi, A. F. 2022. Antibiofilm and Antimicrobial Effectiveness of Chlorhexidine Hexametaphosphate Nanoparticles as a Coating for Orthodontic Miniscrews. *Pakistan Journal of Medical & Health Sciences,* 16(03), 626-626.

Hodin, M. W. 2023. Antimicrobial Resistance: A Major Threat To The Promise Of Healthy Aging. *Health Affairs Forefront.*

Jailani, A., Ahmed, B., Lee, J. H., & Lee, J. 2022. Inhibition of Agrobacterium tumefaciens growth and biofilm formation by tannic acid. Biomedicines, 10(7), 1619.

Jiang, X., Lin, A., Li, S., Shi, Y., Zhou, F., Felix Gomez, G. G., ... & Huang, R. 2022. Effects of artificial honey and epigallocatechin-3-gallate on streptococcus pyogenes. *BMC microbiology*, 22(1), 207.

Jiao, Y., Tay, F. R., Niu, L. na, & Chen, J. hua. 2019. Advancing antimicrobial strategies for managing oral biofilm infections. In International Journal of Oral Science (Vol. 11, Issue 3). *Sichuan University Press.*

Johnston, W., Ware, A., Kuiters, W. F., Delaney, C., Brown, J. L., Hagen, S., ... & Kean, R. 2023. In vitro bacterial vaginosis biofilm community manipulation using endolysin therapy. Biofilm, 5, 100101.

Jungbauer, G., Favaro, L., Müller, S., Sculean, A., & Eick, S. 2022. The in-vitro activity of a cold atmospheric plasma device utilizing ambient air against bacteria and biofilms associated with periodontal or peri-implant Diseases. *Antibiotics,* 11(6), 752.

Kamini, P., Premamalini, T., Karthika, K., & Bavadharani, S. 2022. Antifungal susceptibility profile and biofilm production of Candida spp. isolated from catheterised patients with urinary tract infection. *Journal of The Academy of Clinical Microbiologists*, 24(2), 76.

Kim, E. J., Hyun, J. E., Kang, Y. H., Baek, S. J., & Hwang, C. Y. 2022. In vitro antibacterial and antibiofilm effects of cold atmospheric microwave plasma against Pseudomonas aeruginosa causing canine skin and ear infections. *Veterinary Dermatology,* 33(1), 29-e10.

Kırmusaoğlu, S. 2019. The Methods for Detection of Biofilm and Screening Antibiofilm Activity of Agents. In Antimicrobials, Antibiotic Resistance, Antibiofilm Strategies and Activity Methods.

Kragh, K. N., Tolker-Nielsen, T., & Lichtenberg, M. 2023. The non-attached biofilm aggregate. *Communications Biology*, *6*(1), 898.

Li, Y., Du, J., Huang, S., Wang, S., Wang, Y., Lei, L., ... & Huang, X. 2022. Antimicrobial Photodynamic Effect of Cross-Kingdom Microorganisms with Toluidine Blue O and Potassium Iodide. *International Journal of Molecular Sciences*, 23(19), 11373.

Liaqat, I., & Farooq, M. 2023. 10. A review on the role of bacteria based biofilm inhibitors. *Pure and Applied Biology* (PAB), 12(1), 567-582.

Liaqat, I., & Farooq, M. (2023). 10. A review on the role of bacteria based biofilm inhibitors. *Pure and Applied Biology* (PAB), 12(1), 567-582.

Lichtenberg, M., Kragh, K. N., Fritz, B., Kirkegaard, J. B., Tolker-Nielsen, T., & Bjarnsholt, T. 2022. Cyclic-di-GMP signaling controls metabolic activity in Pseudomonas aeruginosa. *Cell reports,* 41(3), 111515.

Liu, H., Hu, Z., Li, M., Yang, Y., Lu, S., & Rao, X. 2023. Therapeutic potential of bacteriophage endolysins for infections caused by Gram-positive bacteria. Journal of biomedical science, 30(1), 29.

Logambal, S., Thilagavathi, T., Chandrasekar, M., Inmozhi, C., Kedi, P. B. E., Bassyouni, F. A., ... & Kaviyarasu, K. 2023. Synthesis and antimicrobial activity of silver nanoparticles: incorporated couroupita guianensis flower petal extract for biomedical applications. *Journal of King Saud University-Science,* 35(1), 102455.

Ly, K. L., Colon-Ascanio, M., Ou, J., Wang, H., Lee, S. W., Wang, Y., ... & Luo, X. 2023. Dissolvable alginate hydrogel-based biofilm microreactors for antibiotic susceptibility assays. Biofilm, 5, 100103.

Madivoli, E. S., Kareru, P. G., Gachanja, A. N., Makhanu, D. S., & Mugo, S. M. 2022. Cellulose-based hybrid nanoarchitectonics with silver nanoparticles: Characterization and Antimicrobial potency. *Journal of Inorganic and Organometallic Polymers and Materials,* 32(3), 854-863.

Magalhães, R. P., Vieira, T. F., Fernandes, H. S., Melo, A., Simões, M., & Sousa, S. F. 2020. The Biofilms Structural Database.

Mgomi, F. C., Yang, Y. R., Cheng, G., & Yang, Z. Q. 2023. Lactic acid bacteria biofilms and their antimicrobial potential against pathogenic microorganisms. Biofilm, 100118.

Michaelis, C., & Grohmann, E. 2023. Horizontal gene transfer of antibiotic resistance genes in biofilms. Antibiotics, 12(2), 328.

Nadagouda, M. N., Vijayasarathy, P., Sin, A., Nam, H., Khan, S., Parambath, J. B., ... & Han, C. 2022. Antimicrobial activity of quaternary ammonium salts: structure-activity relationship. *Medicinal Chemistry Research*, 31(10), 1663-1678.

Nadar, S., Khan, T., Patching, S. G., & Omri, A. 2022. Development of Antibiofilm Therapeutics Strategies to Overcome Antimicrobial Drug Resistance. In Microorganisms (Vol. 10, Issue 2). MDPI.

Namburu, J. R., Santosh, A. B. R., Poosarla, C. S., Manthapuri, S., Pinnaka, M., & Baddam, V. R. R. 2022. Streptococcus Mutans-Specific Antimicrobial Peptide C16G2-Mediated Caries Prevention: A Review. Frontiers in Dentistry.

Nguyen, T. L. A., & Bhattacharya, D. 2022. Antimicrobial activity of quercetin: an approach to its mechanistic principle. Molecules, 27(8), 2494.

Ning, Y., Hou, L., Ma, M., Li, M., Zhao, Z., Zhang, D., ... & Jia, Y. 2022. Synergistic antibacterial mechanism of sucrose laurate combined with nisin against Staphylococcus aureus and its application in milk beverage. LWT, 158, 113145.

Osimitz, T. G., & Droege, W. 2022. Adverse Outcome Pathway for Antimicrobial Quaternary Ammonium Compounds. Journal of Toxicology and Environmental Health, Part A, 85(12), 494-510.

Pakkulnan, R., Thonglao, N., & Chareonsudjai, S. 2023. DNase I and chitosan enhance efficacy of ceftazidime to eradicate Burkholderia pseudomallei biofilm cells. *Scientific Reports,* 13(1), 1059.

Phuket, T. R. N., Charoensapsri, W., Amparyup, P., & Imjongjirak, C. 2023. Antibacterial activity and immunomodulatory role of a proline-rich antimicrobial peptide SpPR-AMP1 against Vibrio campbellii infection in shrimp Litopenaeus vannamei. *Fish & Shellfish Immunology,* 132, 108479.

Pourhajibagher, M., Keshavarz Valian, N., & Bahador, A. 2022. Theranostic nanoplatforms of emodin-chitosan with blue laser light on enhancing the anti-biofilm activity of photodynamic therapy against Streptococcus mutans biofilms on the enamel surface. *BMC microbiology,* 22(1), 1-16.

Qian, Y., Guo, Y., Shen, J., Qin, Y., & Li, Y. Y. 2022. Biofilm growth characterization and treatment performance in a single stage partial nitritation/anammox process with a biofilm carrier. Water Research, 217, 118437.

Ramírez-Larrota, J. S., & Eckhard, U. 2022. An introduction to bacterial biofilms and their proteases, and their roles in host infection and immune evasion. *Biomolecules,* 12(2), 306.

Rath, S., Bal, S. C. B., & Dubey, D. 2021. Oral Biofilm: Development Mechanism, Multidrug Resistance, and Their Effective Management with Novel Techniques. *Rambam Maimonides Medical Journal,* 12(1)

Repac Antić, D., Parčina, M., Gobin, I., & Petković Didović, M. 2022. Chelation in antibacterial drugs: From nitroxoline to cefiderocol and beyond. *Antibiotics,* 11(8), 1105.

Roy, P. K., Song, M. G., & Park, S. Y. 2022. Impact of Quercetin against Salmonella Typhimurium Biofilm Formation on Food–Contact Surfaces and Molecular Mechanism Pattern. Foods, 11(7), 977.

Roy, R., Tiwari, M., Donelli, G., & Tiwari, V. 2018. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence, 9(1), 522–554.

Sabino, C.P., Ribeiro, M.S., Wainwright, M., dos Anjos, C., Sellera, F.P., Dropa, M., Nunes, N.B., Brancini, G.T.P., Braga, G.U.L., Arana-Chavez, V.E., Freitas, R.O., Lincopan, N. and Baptista, M.S. 2023. The Biochemical Mechanisms of Antimicrobial Photodynamic Therapy†. *Photochem Photobiol,* 99: 742-750.

Saverina, E. A., Frolov, N. A., Kamanina, O. A., Arlyapov, V. A., Vereshchagin, A. N., & Ananikov, V. P. 2023. From Antibacterial to Antibiofilm Targeting: An Emerging Paradigm Shift in the Development of Quaternary Ammonium Compounds (QACs). ACS Infectious Diseases, 9(3), 394-422.

Selvi, S., Polat, R., ÇAKILCIOĞLU, U., Celep, F., Dirmenci, T., & ERTUĞ, Z. F. 2022. An ethnobotanical review on medicinal plants of the Lamiaceae family in Turkey. *Turkish Journal of Botany,* 46(4), 283-332.

Shimizu, T., Aritoshi, T., Beatty, J. T., & Masuda, T. 2022. Persulfide-responsive transcription factor SqrR regulates gene transfer and biofilm formation via the metabolic modulation of cyclic di-GMP in Rhodobacter capsulatus. *Microorganisms,* 10(5), 908.

Sleutel, M., Pradhan, B., Volkov, A. N., & Remaut, H. 2023. Structural analysis and architectural principles of the bacterial amyloid curli. *Nature Communications*, 14(1), 2822.

Soheili, M., Alinaghipour, A., & Salami, M. (2022). Good bacteria, oxidative stress and neurological disorders: Possible therapeutical considerations. *Life Sciences*, 120605.

Sondhi, S. 2023. Application of biosurfactant as an emulsifying agent. In Applications of Next Generation Biosurfactants in the Food Sector (pp. 43-56). Academic Press.

Su, X., Luo, J., Wang, Y., Dong, J., & Tian, Y. 2023. Biofouling mitigation by D-tyrosine in membrane bioreactor: Short-term performance. *Journal of Environmental Chemical Engineering,* 11(2), 109554.

Subhadra, B. 2022. Biofilm Composition and Applications. Coatings, 12(7), 1026.

Sudhakaran, G., Guru, A., Haridevamuthu, B., Murugan, R., Arshad, A., & Arockiaraj, J. 2022. Molecular properties of postbiotics and their role in controlling aquaculture diseases. *Aquaculture Research,* 53(9), 3257-3273.

Sun, N., Zhang, X., Hou, Y., & Zhong, T. 2022. A novel non-invasive biomarker based on oral microbiome dysbiosis for detection of Community-Acquired Pneumonia. bioRxiv, 2022-10.

Trilokesh, C., Harish, B. S., & Uppuluri, K. B. 2023. The antibiofilm potential of a heteropolysaccharide produced and characterized from the isolated marine bacterium Glutamicibacter nicotianae BPM30. *Preparative Biochemistry & Biotechnology*, 1-9.

Vargas, E. L. G., Almeida, F. A. D., de Freitas, L. L., Pinto, U. M., & Vanetti, M. C. D. 2022. Furanone and phytol influence metabolic phenotypes regulated by acyl-homoserine lactone in Salmonella. *Brazilian Journal of Microbiology*, 1-12.

Vieira-da-Silva, B., & Castanho, M. A. 2022. The structure and matrix dynamics of bacterial biofilms as revealed by antimicrobial peptides' diffusion. Journal of Peptide Science, e3470.

You, R., Kwon, O. Y., Woo, H. J., & Lee, S. H. 2022. Hovenia Monofloral Honey can Attenuate Enterococcus faecalis Mediated Biofilm Formation and Inflammation. *Food Science of Animal Resources,* 42(1), 84.

Yu, L., Li, K., Zhang, J., Jin, H., Saleem, A., Song, Q., ... & Li, P. 2022. Antimicrobial peptides and macromolecules for combating microbial infections: from agents to interfaces. *ACS Applied Bio Materials,* 5(2), 366-393.

Zhang, C., & Yang, M. 2022. Antimicrobial peptides: From design to clinical application. *Antibiotics,* 11(3), 349.

Zhang, K., Li, X., Yu, C., & Wang, Y. (2020). Promising Therapeutic Strategies Against Microbial Biofilm Challenges. In Frontiers in Cellular and Infection Microbiology (Vol. 10). Frontiers Media S.A.

Zhang, P., Wu, S., Li, J., Bu, X., Dong, X., Chen, N.... & Liu, Z. 2022. Dual-sensitive antibacterial peptide nanoparticles prevent dental caries. Theranostics, 12(10), 4818.

Zhao, H., Wang, X., Geng, Z., Liang, N., Li, Q., Hu, X., & Wei, Z. 2022. Dual-function microneedle array for efficient photodynamic therapy with transdermal co-delivered light and photosensitizers. Lab on a Chip, 22(23), 4521-4530.

****