Review Article

Contribution of Biofilm Activity in Development of Antibiotic Resistance - A Global Threat

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ABSTRACT

A microbial community or association known as a biofilm is adhered to various abiotic or biotic surfaces or habitats. In comparison to non-adherent, planktonic cells, biofilms are surface-attached collections of microbial cells wrapped inside an extracellular matrix those are substantially more resistant to antimicrobial treatments. As a result, biofilm-based infections are very complicated to cure. Due to its formation on medical implants within human tissue and inclusion in a variety of dangerous chronic infections, biofilm is a serious issue in the healthcare profession. A matrix of extracellular polymeric materials surrounds the microorganisms in a biofilm, acting as a barrier and resistive to various unfriendly environments like antiseptics, antibiotics, or other hygienic conditions. The development of antibiotic resistance by biofilms, which complicates treatment options and is caused by a variety of physiological, physical, and gene-related variables, is the other major problem with biofilm formation. The high level of recalcitrance that distinguishes biofilm communities comes from a variety of molecular pathways. These mechanisms include, among others, how antimicrobials interact with the elements of biofilm matrix, slower growth rates, and the varied ways in which particular genetic determinants of antibiotic resistance work. Each of these mechanisms by themselves can only partially explain the elevated antibacterial recalcitrance seen in biofilms. It is better to comprehend growth requirements and procedures in order to reduce their production and create managing approaches. This review article's objective is to provide a general overview of how bacterial biofilms contribute to antibiotic resistance while emphasising strategies for reducing their formation.

Keywords: Extracellular matrix, extracellular polymeric substance, antibiotic resistance, quorum sensing.

INTRODUCTION

After the 1938 discovery of penicillin, bacterial infections were effectively controlled by antibiotics[1]. Antimicrobial resistance is growing upswing, which poses a risk to the potential health benefits that antibiotics provide, and it is highlighted as a worldwide concern[2]. Getting rid of the implants that own is the only method of treating infections brought on by biofilms, which are unresponsive microbial communities which often colonise and continue to expand on the outer surface of medical implants like sutures, intravenous, and dental implants. This is expensive for the affected person and can result in mental illness[3]. An extracellular matrix (ECM), which is constructed of extracellular polymeric compounds, is where cells are attached and protected in a biofilm, which is a consortium of microorganisms[4], which contains extracellular DNA, amyloid precursor protein proteins, proteins, and other polymers formed by bacteria (e-DNA)[5,6]. The biofilm formation is a well-regulated multiple step process that includes the adhesion of macro and micro molecules to the surfaces, the adhesion of bacteria to the surfaces and the discharge of extracellular polymeric substances (EPS), the formation of colonies, and also biofilm mutation. Contrary to planktonic bacteria, the respiration rate of bacterial communities in biofilms has changed as evidenced by elevated ratio of EPS production, the inhibition or activation of certain genes connected to biofilm construction, as well as reduced growth rates[7]. The synthesis of glucosamine, extracellular DNA (eDNA), lipids, nucleic acids, phospholipids, polysaccharides, and intermolecular interactions ultimately depend on EPS, which are made consisting of proteins, cellulose, poly-N-acetyl, alginates, extracellular teichoic acid, and many other chemical compounds[8,9]. In addition to protecting the bacterium from alterations in pH, osmotic potential, nutritional shortage, mechanical stresses, and shear forces[10,11], biofilms also shelter them from antibacterial drugs and the host's immune cells[12]. Bacteria are given an additional layer of defence by the biofilm matrix, enabling them to resist, not only exposure to the elements but also antibiotics. This results in the creation of bacteria that are multi drug resistant, extensively drug-resistant, even consciously resistant to drug.

Alginate acts as a physical barrier and significantly stabilizes the gluconeogenesis of bacteria in established biofilms, making it difficult to eradicate bacteria from biofilms. New treatment options are urgently needed as

bacterial infections that are highly multi-drug resistant continue to spread[13]. In this review, emerging techniques are examined in relation to dodging, already-in-place resistance mechanisms. Anti microbial resistance is a worldwide ecological catastrophe that several regulatory authorities have identified as a global health threat. As a result, the optimism regarding the control of infection that emerged after the discovery of antibiotics has come to an end, and also new infection control measurements are extremely needed. In order to inspire new technologies to fight various pathogenic bacteria, this overview presents existing obstacles, various alternative treatments, and future prospects.

1. BIOFILM FORMATION:

There are significant variations across bacterial species during the biofilm production stage, which has been seen in practically all bacterial species. Additionally, biofilms protect bacterial invaders from immune system of the host by reducing protease activity and the host immune system[14,15] or by increasing their resistance to commonly used antibiotics[16,17,18]. Congenital and acquired microbial resistance, metabolic state, oxygen and nutrient accessibility, biofilm form and composition, and other elements are known to play a huge role in the development of resistance. According to published research, Pseudomonas aeruginosa biofilm was particularly resistant to tobramycin due to its mucoid structure[19]. Some antibiotics are also unresponsive to cells that are actively developing or dividing, whereas cells that are growing very slowly with limited resources, like cells in the stationary phase, may be tolerant to antibiotics like - lactams. The extracellular biofilm matrix does not always operate as a protective film for antimicrobials, but it does shield the bacterial cells from outside stresses. Chemical or mechanical stressors might cause the biofilm cells to disperse. Anderl et al. proposed that ampicillin was capable of penetrate the Klebsiella pneumoniae strain's -lactamase deficient biofilm but was unable to do so with the strain's wild type K. pneumoniae[20].
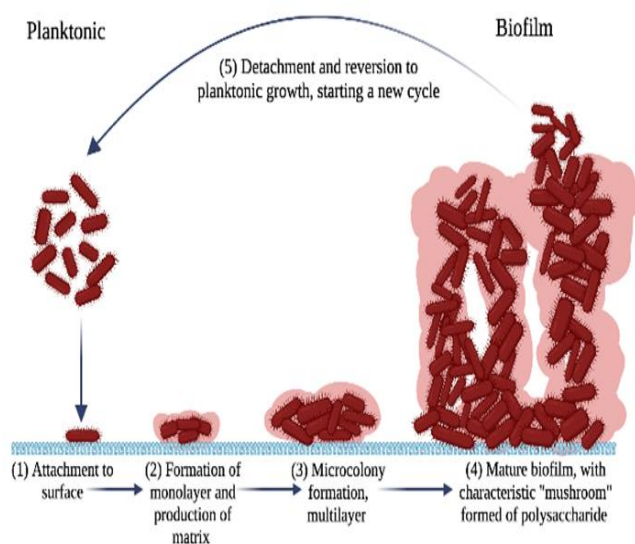
Figure 1: Depicting diagram of the biofilm development

There are five effective stages, that can be identified in the biofilm production: initial attachment which is reversible (stage-1), irreversible attachment (stages-2,3), maturation (stage-4), and dispersion (stage-5) (as shown in Fig. 1). The beginning, which is still reversible at this point, occurs when the migrating planktonic bacteria come into initial contact with the surface.

Development of biofilm is a multiple step process and this process begins with the initial attachment which is irreversible, of the bacteria to the substrate are followed by their colonization, throughout the whole expression changes of their genes and also proteins take place, and an exponential growth phase. Exopolysaccharides and the development of water channels facilitate the nutrient availability, which promotes biofilm maturation[21]. In the end, environmental conditions cause the cells to separate from their surfaces, which causes the biofilm to grow once more on fresh surfaces.

The planktonic cell's initial adhesion towards the surfaces, subsequently followed by the cell separation, adulthood, EPS secretion, and biofilm dispersion, all are the stages that take place during biofilm formation[22]. Three primary processes are there that can be summed up as follows: reversible adherence to the surface which are followed by bacterium division and creation of both extracellular matrix and as well as, ultimately matrix breakdown and bacterial dispersion[23]. While many species depend on quorum sensing (QS) to coordinate the creation of biofilms, this regulatory mechanism may not be the main one because it only acts as a checkpoint as the biofilm develops[22].

1. **Reversible or initial attachment —** An important transition from planktonic life towards the biofilm phase is represented by bacterial surface adhesion[24]. Planktonic bacteria interact with a prepared surface to form reversible adhesion. Although van der Waals force, electrostatic interaction, and hydrophobic contacts are all present, the interaction is relatively weak. According to some reports, the attachment will work best on rough, hydrophobic, and organically covered surfaces[25]. The contact among bacteria and also the surface of adhesion is strengthened by bacterial features including fimbriae, pili, and flagella. At this stage, bacteria and their attached cell appendages either commit to living in biofilms or depart the surface and resume their planktonic state[26].

2. **Permanent Attachment —** By generating extracellular polymeric substances (EPS) that interact with surface components and/or receptor-specific ligands present on pili, fimbriae, and fibrillae, or both, loosely bound organisms strengthen the attachment process at this stage[27,28]. When microorganisms adhere to predisposed and permissive surfaces, an irreversible adhesion continues, and the cell begins to accumulate as multi layered cell clusters[29]. According to current studies, the beginning of the creation of a biofilm is a

layer of EPS in which microbial cells are swarmed upon its surface.

3. **Micro colony Construction —** Micro colonies, which are the fundamental building blocks of biofilm and are formed when microbial cells buried in the extracellular matrix go through coordinated community expansion, are differentiated by channels with various unique micro habitats. Numerous bacteria will then emerge and release polymeric compounds that can function as a "glue" to fix micro-organism on various surfaces when cells are firmly adhered to conductive surfaces. Micro colonies are created as a result of these successive occurrences.

4. **Biofilm Synthesis —** A biofilm may establish into spatially well-arranged, 3D (three-dimensional) mature biofilm structures[30] like mushroom- or tower-like formation interspersed with fluid-filled channels in which nutrients, O2 (oxygen), as well as diffusion of other necessary substances and circulated in each microenvironment[31] if the circumstances are suitable for adequate growth and differentiation (Fig.1). By releasing density-dependent chemical signals, bacterial populations embedded inside a self-generated extracellular matrix coordinate their behaviour to form biofilms[32]. Quorum sensing, a signaling system, is employed to coordinate and coordinate group activities such virulence factor secretion , biofilm formation. Quorum sensing coordinately activates the biofilm's maturation and disintegration[32]. In general, cell adherence and detachment from biofilm are greatly influenced by cell-to-cell signaling pathway[33].

5. **Dispersion of Biofilms —** A cyclical process of biofilm formation involves the separation of bacterial cells from the mature biofilm including their return to the planktonic state (fig. 1). The scattered bacterial cells will then seek out new surfaces to attach to it and begin a new round of biofilm production. In this phase, microbial cells will choose whether to coexist or "fall apart" based on environmental stimuli[34]. Biofilm cells can be separated from other cells that are actively growing or from a poor environment. They can also communicate or get rid of aggregates. Microorganisms have been said to seek out new settings as a result of nutrition constraint[34].

Table 1: As per this review description of certain antibiotics and their primary methods of action.

Classification of Antibiotics	Examples	Primary methods of action
Inhibitors of cell membrane synthesis		
β-Lactams	Penicillins (like ampicillin and penicillin G), cephalosporins (such as cefotaxime and cefazolin), and carbapenems (meropenem, imipenem)	By preventing transpeptidases, stop peptidoglycan from being transpeptided, penicillin-binding proteins
Glycopeptides	Vancomycin	Attach with the terminal D-Ala-D-Ala residues in peptidoglycan subunits which prohibit transpeptidation.
Disrupters of the cell membrane		
Lipopeptides	Daptomycin	Depolarization of the cell membrane
Polymixins	Colistin, polymyxin B	Rupture the outer membrane by binding LPS
Inhibitors of nucleic acid synthesis		
Quinolones	Fluoroquinolones like ciprofloxacin, ofloxacin, norfloxacin	Prevent the replication process of DNA by complex assemble with topoisomerase IV or DNA, DNA gyrase
Rifamycins	Rifampin	RNA polymerase inhibition
Sulfonamides	Sulfamethoxazole	Ultimately, inhibit dihydropteroate synthase from synthesizing THFa.
Inhibitors of Di-hydrofolate reductase (DHFR)	Trimethoprim	Inhibit DHFR to prevent THFa formation

Inhibitors of protein synthesis		
Aminoglycosides	Amikacin, kanamycin, gentamicin, tobramycin	Entangle with 30S ribosomal subunit's 16S rRNA and obstruct various protein synthesis prospects.
Tetracyclines	Doxycycline, tetracycline	Inhibit aminoacyl-tRNA to from attachment of the ribosomal A sites, when 30S ribosomal subunit's 16S rRNA bonding happens.
Macrolides	Clarithromycin and azithromycin	Binds to the 50S ribosomal subunit's 23S rRNA and peptide chains extension is prevented.
Oxazolidinones	Linezolid	Binds with the 50S ribosomal subunit's 23S rRNA and prevent the translational translation.
Chloramphenicol	Chloramphenicol	Binds to the 50S ribosomal subunit's 23S rRNA and prevent the extension of peptide chains.
anti-steroid medications	Fusidic acid	Restrict the elongation factor G function.

## 2. MODULATION IN BIOFILM FORMATION AFTER ANTIBIOTIC APPLICATION :

A crucial challenge in biofilms improved survival and tolerance to both the environmental and chemical stresses (such as antibiotics) is the extracellular polysaccharide matrix's protection[35]. When compared with their planktonic counterparts, bacterial biofilms are 10 to 1,000 times fewer susceptible to some antimicrobial treatments[36,40]. A number of several factors, including (i) a poor antibiotic penetration into polysaccharide matrix, (ii) the arbitrary presence of cells with a resistant phenotype (referred to as "persisters"), as well as (iii) the appearance between either non-growing cells or cells which are triggered stress responses under unfavourable chemical conditions within the biofilm matrix[37], contribute to this reduced susceptibility. These defence mechanisms work in concert with those causing traditional resistance brought on by the expression of, either genes (ARGs) in bacterium genomes or extrachromosomal components, increasing biofilms' overall resistance to antimicrobial substances. For instance, because those ß-lactamases inactivated the ß - lactam antibiotic, including the use of ampicillin, they provided enhanced protection in biofilms. Additionally, exposure to medicines like imipenem dramatically activated the ampC gene in Pseudomonas aeruginosa biofilms[38]. Additionally, it has been discovered that Escherichia coli and P. aeruginosa both produce biofilms as a defensive response to antibiotic presence at sub-inhibitory concentration range of amino glycosides[39,41]. Similar findings were reported by who discovered that the concentrations of tetracycline (sub-inhibitory) and cephradine promote the transmission of both the pB10 plasmid among with such biofilm biomass (P. aeruginosa or even E. coli) at the ratio of 2–5 times quicker than without the treatment of antibiotics. Since most bacterial pathogens frequently form biofilms, the increased antibiotic biofilms resistance is a critical concern for human health factors[42,43,44]. Many chronic infections have been associated with biofilm growth on either the natural surfaces (such as the lungs or teeth) or foreign body implants (e.g., prosthetic heart valves, pacemakers, catheters).

CONTRIBUTION OF BIOFILM IN DEVELOPMENTAL OF ANTIBIOTIC RESISTANCE :

Mechanism —

When bactericidal or bacteriostatic antimicrobial agents are present, resistant bacteria as well as microorganisms can thrive at a concentration where typically prevents it. The MIC (minimum inhibitory concentration) in which the lowest concentration and at which an antimicrobial drug inhibits microbe growth, is used to assess the planktonic cultures resistance. Also resistance can be inherent, depending upon a cell's innate characteristics and wild type genes, or it can be acquired, contributing to alteration or gene mutations which are resistant to antibiotics[45,46]. The two primary reasons of infectious biofilm resistance to anti bacterial treatment, that are inadequate antibacterial penetration into biofilms and inherent to antibacterial resistance. Initially, planktonic or suspended antibacterial sensitive bacteria are eliminated, however only biofilm bacteria may reach and be killed inside of a biofilm and the inner ones cannot. Antibiotic-resistant bacteria, however, are neither eliminated by plankton nor by biofilm. Second, inadequate antimicrobial penetration across a biofilm to its depth is a common cause of resistance. Inadequate antimicrobial diffusion as well as adsorption on the EPS protective matrix that was self-produced causes poor penetration. The EPS matrix transports all the nutrients and metabolic waste substance through water channels (the pH of pathogenic biofilms; about 5-9 is less than physiological pH levels) (Fig. 2)[47,48].
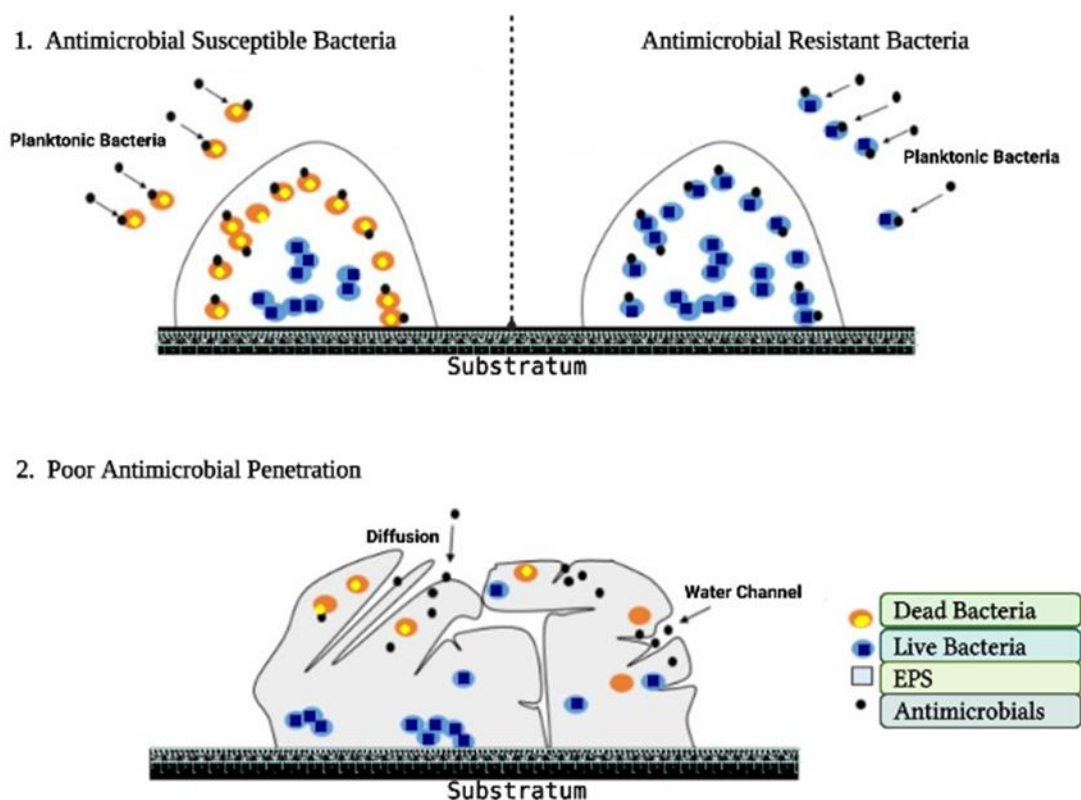
Figure 2: Inherent resistance of antimicrobials and their inadequate penetration of biofilms; **1** Antimicrobial-resistant bacteria are not killed outside of a biofilm or in a planktonic form, however antimicrobial-susceptible bacteria are killed planktonically; **2** Antimicrobials penetrate a biofilm poorly due to low adsorption into extracellular polymeric substances (EPS) protective matrix but rather poor antimicrobials diffusion.

Because of the relative low permeability of the outer membrane of gram negative bacteria, they are much more susceptible to vancomycin like other antibiotics than gram positive bacterial cell[49]. Quorum sensing has a crucial regulatory role in the development of biofilm. It controls how gram negative gram positive and bacteria which produce biofilm. A quorum sensing mechanism controls the acyl homoserine lactone biofilm, which is created by gram negative bacteria and it is made up of the matching receptors signal molecules. For instance, Pseudomonas aeruginosa has two quorum sensing signalling systems: rhlI/rhlI and lasl/lasR. The lasl/rhlI and lasR/rhlR genes are respectively encode several signal molecule receptors and also synthetases. The signal molecules synthesis rises together with bacterial population density. The signal molecule binds to its corresponding receptor and once it reaches a certain threshold it switches to activate. This activation then activates transcriptional regulators necessary for the alginates synthesising, toxic factors, and extracellular polysaccharides, which results in a biofilm formation[50].

By utilizing a signal molecule called oligopeptides, the quorum sensing mechanism of gramme positive bacteria regulates their biofilm. It can be recognised after modification by two-component sensing proteins, whose phosphorylation and dephosphorylation controls the expression of their target genomes and developed biofilms.

Several species of bacteria have several quorum sensing systems and signalling molecules for various oligopeptides. For instance, the responsive regulatory protein and histidine protein kinase are two component system in Streptococcus, meanwhile the quorum sensing system substantially conserved in Staphylococcus aureus[51].

Microorganisms can endure the presence of a single bactericidal antimicrobial agent due to their antimicrobial agent tolerance, and they are unable to grow or reproduce (the minimum concentration of a bactericidal antimicrobial agent at which it can kill over than 99.9% of cells in culture). Toxin actions like MazF and RelE from toxin - antitoxin (TA) modules have been related to the Persister cells formation. Persister cells are highly antibiotic-tolerant, sub-population of bacteria that have not undergone genetic modification to achieve this status. Because most cells are killed by antibiotics, the biofilm of persister cells which is responsible for persistent infections to be resistant and whenever antibiotic level decreases, they continue to be viable as well as repopulate biofilms[52].

Resistance develops as a result of genetic alterations that prevent antibiotics from working as intended. Cells that are resistant to antibiotics thrive, whereas persistent cells lay dormant and are unable to proliferate. Low metabolic activity is essential for persister cell survival, and ongoing research with metabolic regulators has also shown a correlation between increased persistence and decreased

metabolic activity[53]. This shows that the key factor reducing the susceptibility of biofilm against antibiotics is the much slower growth rate linked towards bacterial stress response and typical of the inner structure of biofilms[54]. Several factors including substance delivery, high density of cell, a rise in the number of resistant mutants, molecular interactions, persistent cells, and efflux pump, contribution against various antimicrobial's biofilm resistance. Several resistance mechanisms over there, such as constrained medication absorption and increase biofilm resistance. For example, oxacillin, vancomycin, as well as cefotaxime penetration were reduced by the biofilms which are generated by susceptible or intermediate types of S. aureus[55]. Halogenated phenazines HPs have frequently demonstrated significant eradication action against previously introduced methicillin resistant Staphylococcus aureus (MRSA) with low toxicity against human cells. Halogenated phenazines govern processes that have been demonstrated to favour bacterial cells across a mammalian cell in the eradication process reduce non-hemolytic metal (II), which dependent upon mechanism action[56].

INVOLVEMENT OF BIOFILM IN BACTERIAL PATHOGENESIS:

The pathogenicity of bacteria that form biofilms can be attributed to a wide range of circumstances. Biofilms emit extracellular chemicals that is with the aid of quorum sensing and alter the gene expression of various virulence factors. Moreover, the bacteria in biofilm increase the frequency of maturation to increase β-lactamase activity, increase efflux pump activity and avoid host defences, and swap plasmids for genetic transmission for virulence factors and also resistant to antibiotics, by which mutation frequency increases. The pathogenicity of biofilms that provide a barrier of protection and have minimal immune cell as well as antibiotic penetration is also influenced by the extracellular matrix characteristics[57]. Antibiotics' minimal inhibitory concentration (MIC) is known to be efficient against planktonic bacteria but ineffective against bacteria found in biofilm. As a result, antibiotics must be employed at a minimal biofilm eradication concentrations (MBEC) that can be up to thousand times higher than that planktonic bacteria[58]. The degradation of metabolic substances occurs in biofilms due to biochemical and physiological gradients of oxygen and nutrients, which also serve as a medium for cell doubling and dormancy. When various antimicrobial drugs are present, cells may also enter a state of dormancy, which is followed by a reversal of dormancy and the release of an antitoxin that renders the cells inactive[59]. Many antibiotics target metabolically active cells, cells that divide quickly, cells that may enable biofilms develop antibiotic resistance after being exposed to stressors, and they also target the delayed growth state of latent cells[60]. Dental caries, cystic fibrosis, ocular implant infection, eardrum infection, native valve endocarditis and urinary tract infections and also osteomyelitis are just a few persistent illnesses that are brought on by biofilms. Many pathogens, such as Streptococcus pneumoniae and Haemophilus influenzae in chronic otitis media, Pseudomonas aeruginosa in cystic fibrosis pneumonia, and enteropathogenic Escherichia coli caused recurrent urinary tract infections that all are linked to the development of biofilm.

Biofilms dramatically improved pathogens' capacity to resist both drugs and host defences. They are interested in the pathophysiology and clinical appearance of various illnesses[61]. The number of infections brought on by biofilms estimation range must be between 65% by the Center for Disease Control and Prevention and 80% by the National Institutes of Health, particularly in the Developed World. Children's gingivitis, middle ear infections, and also the development of typical dental plaque are all brought which depends upon different common diseases such Staphylococcus aureus catheter infections, Escherichia coli urinary tract infections, and *Haemophilus influenza*[62]. P. aeruginosa causes various biofilm infections in cystic fibrosis patients, while Staphylococcus aureus causes endocarditis, which is fatal and morbid. 8–10% of hospitalised patients are susceptible to infections because opportunistic pathogenic microorganisms like Pseudomonas aeruginosa and Staphylococcus aureus create chronic biofilm-based illnesses in their host.

Although conventional adjuvant therapies like hydrogen peroxide, sodium hypochlorite, or povidone-iodine previously eradicated Staphylococcus aureus biofilms , biofilms can survive under various conditions including minor pH changes (either rise or decrease)[63]. The MBEC of acetic acid, on the other hand, was found in an in-vitro investigation to have exceeded its safety threshold after 20 minutes of therapy, making it an unacceptable clinical option[64]. As a result, it is difficult to apply various chemical adjuvant therapies since biofilms can live in a variety of conditions, including those with severe pH levels. Moreover, a foreign body implant without a blood supply, which limits the capability of antibiotics and as well as immune cells to effectively reach an infection site[65].

BIOFILM PRODUCING ORGANISM CAUSES HEALTH CONCERNS INCLUDING INFECTIONS IN THE PRESENCE OF ANTIBIOTICS:

Bacterial infections have been a threat to human life since it began. Biofilm growth in the food business is a severe public health hazard due to growing fundamental concerns.

Numerous biofilms contains various harmful bacterial or fungal species that occasionally target only the immunocompromised (i.e. HIV, organ transplant beneficiaries, or oncology patients etc.). These bacteria can result in food poisoning (*Bacillus cereus* and staphylococcus aureus), gastroenteritis (*Escherichia coli* and *Salmonella enterica*), as well as systemic illnesses (Listeria monocytogenes, *Escherichia coli*)[66]. Bacillus subtilis produces a secreted peptide antibiotic identified as YIT toxin and biofilm-resistant proteins. A mutant with deficient resistance genes was shown to create biofilms[67]. Toxin-Antitoxin (TA) systems have a role in persister cell and biofilm development, and these systems' secondary messenger, 3', 5'-cyclic diguanylic acid, may be a key

regulator of the transition from planktonic to biofilm life in response to stress.

Some food processing facilities have biofilms that can release poisons. From there, food matrix can become tainted, resulting in one intoxication or many. Depending on several types of bacterial species involved, the existence of biofilms in food factories puts human health at risk in each scenario. Depending on the type of factory, milk, water and other pipeline liquids, tables, reverse osmosis membranes, contact surfaces, pasteurizer plates, packaging, animal carcasses, dispensing tubing, employee gloves, storage silos for additives and raw materials, etc. are among the primary sites for biofilm development[68].

As biofilm-based infections can tolerate antibiotic dosages that would often kill free-swimming planktonic cells, they are challenging to treat. Bacterial biofilms have a major impact on patient morbidity and death as a result. Persister cells, stress reactions, and biofilm heterogeneity are only a few of the mechanisms that have been discovered to support antibiotic resistance and tolerance in biofilms, as was covered in this study. Furthermore, it has emerged that genetic factors frequently underlie the basic processes of antibiotic tolerance and resistance in biofilms. For reference, Table 2 provides a non-exhaustive list of the various genes which have been linked to biofilm resistance.

Table 2: Examples of how antibiotic resistance and tolerance genes are specific to biofilms.

Responsible Gene(s)	Genetic Product(s)	Proposed mechanism for protection	Applicable Antibiotics	References
Pseudomonas aeruginosa				
brlR	Transcriptional Activation	Increased expression of multiple drug efflux pumps	Chloramphenicol, kanamycin, tetracycline, trimethoprim, tobramycin, norfloxacin, and trimethoprim	69,70,71,72
sagS	Two Component Hybrid	BrlR activation through stimulating enhanced c-di-GMP levels	Norfloxacin, tobramycin	73,74,75
ndvB	Glucosyltransferase	ethanol oxidation genes are up-regulated and antibiotics are sequestered.	Ciprofloxacin, gentamicin, and tobramycin	7,67,778
exaA, pqqC, erbR	Oxidation of Ethanol players	Unknown	Tobramycin	79
PA1875-1877	Biofilm Specific Antibiotic Efflux Pump	Antibiotic Efflux from the Cell	Ciprofloxacin, gentamicin, and tobramycin	80
tssC1, hcp1	Type VI Secretion Components	Unknown	Ciprofloxacin, gentamicin, and tobramycin	81
PA0756-0757	Two Component System	Unknown	Gentamicin with tobramycin	82
PA2070	TonB Component Receptor	Unknown	Gentamicin with tobramycin	82
PA5033	Hypothetical Proteins	Unknown	Gentamicin with tobramycin	82
relA, spoT	Stringent Responsive Players	Increase antioxidant defences while decreasing pro-oxidants	Gentamicin, meropenem, colistin, and ofloxacin	84

Enterococcus faecalis				
epaoX	Glucosyltransferase	Maintaining the integrity of the cell wall	Gentamicin	85
epal	Glucosyltransferase	Unknown— perhaps promotion of DNA release	Daptomycin	85
gelE	Gelatinase	Unknown— likely regulation of gelE release	Daptomycin, gentamicin, and linezolid	85
fsrA, fsrC	Quorum-Sensing Players	Unknown	Daptomycin, gentamicin, and linezolid	85
Streptococcus mutans				
dltABCD	Enzymes involved in teichoic acid's D-alanylation	Decrease in the cell wall's negative charge	Gentamicin	86
Escherichia coli				
rapA	Helicase like proteins	YhcQ and exopolysaccharide synthesis both are upregulated.	Norfloxacin, gentamicin, chloramphenicol, and penicillin G	87
yafQ	Toxin	Formation of persister cells	Cefazolin, tobramycin	88

COMPLICATIONS WITH ERADICATING BIOFILMS:

Biofilms can endure a variety of antimicrobial substances in both industrial and natural settings. Cells that produce biofilms benefit from structure and gene expression for growth and survival. Biofilms are also heterogeneous in both time and space. The removal of biofilm is challenging for three main reasons (Fig. 3)[89,90].
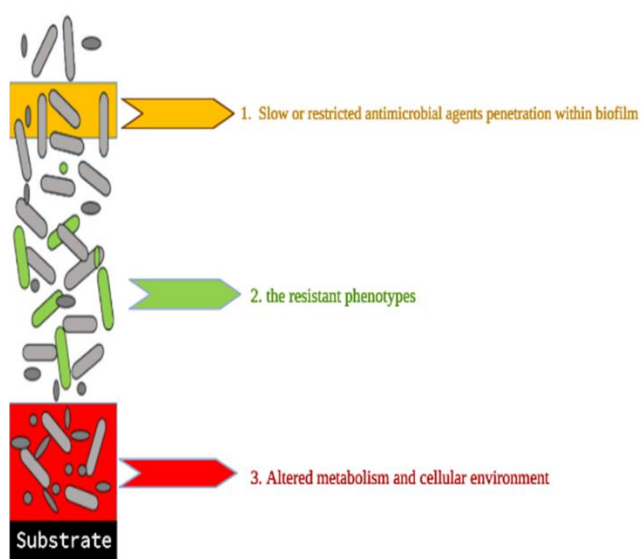
Figure 3: Three hypotheses for how biofilms develop antibiotic resistance

First, different antimicrobial drugs only partially or slowly penetrate biofilm. And the second is the resistant phenotype, which consists of gene transfer and enzymes that degrade antimicrobials. The distinct variances in gene expression between planktonic and sessile cells what cause physiological changes during biofilm development. Third one is altered metabolism and cellular environment. In a biofilm, some cells are starved or in a slow-growing state because of nutritional limitations[91,92].

Most of cells have a protective phenotype and are anoxic, they are less vulnerable to various antimicrobial drugs. They are numerous in deep biofilm and are referred to as persister cells. As a result, these many characteristics make the removal of biofilm challenging and enhance resistance[91,92].

NANOTECHNOLOGY'S INVOLVEMENT IN ERADICATING BIOFILMS:

As per their utilization as a replacement to tackle infections based on multiple drug resistance and biofilm, nanoparticles are of utmost importance[93]. The numerous limits and limitations of conventional treatments can be overcome by their nano-formulations. These formulas are capable of transcending biological barriers. As anti-biofilm and antibacterial metal nanoparticles, green nanoparticles, and many other combinations have been used in the past[94]. Nanoparticles including copper, oxide, silver, zinc, and quantum dots have demonstrated as per potential against biofilms due to their strong antibacterial capabilities[95]. Through raising oxidative stress, promoting cytoplasmic leakage, and denaturing metabolic proteins, nanoparticles carrying reactive oxygen species (ROS) harm bacteria's cel walls are well as cell membranes[96]. It causes altered cell activities and has an impact on bacterial physiological processes[95]. Several in-vivo studies have discussed that

nanoparticles have great compatibility and minimal cell toxicity, making them an effective weapon against a wide range of gram positive and gram negative bacterial strains[97,98]. This results in the potential utilization nanoparticles as a treatment for bacterial illnesses[99].

Kulshrestha and his colleagues reported CaF2-NP's suppressive effect on genes linked with virulence factors, including vicR, ftf, gtfC, comDE, and spaP of S. mutans in addition to showing enzymatic inhibition activity associated with cell adhesion, glucan synthesis, acid production, and quorum sensing also the acid tolerance[100].

QUORUM SENSING'S INVOLVEMENT IN ERADICATING BIOFILMS:

Biofilm synthesis was regulated by those genes which are involved in quorum sensing (QS) signaling. Many inhibitors and substances can interfere with the QS signaling cascade and be utilized as an alternative to conventional therapeutics for the treatment of infections caused by biofilms. Halogenated furanone, which is obtained from the marine algae Delisea pulchra, can be used to disrupt bacterial QS signalling[101]. Acyclic diamine (ADM 3) has recently been shown by Kaur and his colleagues to have greater anti-biofilm activity[102]. Garlic, usnic acid, ginseng, and azithromycin extracts all enhance the inhibitory effects against bacterial and fungal biofilm production[103,104]. Via c-di-GMP-degrading phosphodiesterase simulation, nitric oxide which functions as a signalling molecule that disperses biofilms in species like P. aeruginosa and enhances antimicrobial drugs efficiency[105,106].

Advanced New Innovative Components to Eradicate Biofilms —

By lowering the selective pressure for medication resistance, finding new inhibitors of bacterial biofilm that do not influence bacterial growth may help create new anti-toxin tactics. The most crucial building blocks for creating compounds with various biological properties, such as anti-diabetic, anti-inflammatory, antibacterial, analgesic, anticancer, and anti-HIV, are thiazoles, thiazolidinone derivatives, and their benzofused structures[107].

Against reference strains of gram negative bacteria, a family of 36 novel compounds called 2-(6- phenylimidazo [2, -1-b] [1, 3, 4] thiadiazol-2-yl)- 1H-indoles have been thoroughly created and observed for their anti-biofilm capabilities. The therapeutic benefits of indole compounds as analgesic, antibacterial, antiviral, anti-inflammatory, and anticancer medicines have been extensively discussed[108]. New 1, 2, and 4-oxadiazole compounds have been successfully created and tested as potential novel anti virulence medicines. gram negative and gram positive microorganisms were investigated and their ability to create biofilms was compared[109]. Despite the fact that the imidazo thiadiazole scaffold has a number of biological features that have been described and that its anti-biofilm action has just recently been discovered[110].

FUTURE ASPECTS

Microorganism communities that are adhered to surfaces form biofilms. Planktonic cells have different characteristics from those biofilm-grown cells, and those cells also exhibit greater certain antimicrobial substances resistant. These and other research suggest that biofilm formation is a systematic and controlled developmental process that leads to the emergence of complex organism communities. Because of a variety of factors, including an altered micro environment, the presence of various persister cells from bacteria, inadequate penetration of several antibiotics and adaptive responses, the antibiotic susceptibility of bacteria is lowered in biofilms. To get rid of these resistance, a range of therapies can be applied, including quorum sensing, nanotechnology, and more.

The capacity of present available antibiotics to treat infections that are currently resistant to accessible current therapies may be improved by inhibiting biofilm resistance. To completely understand the antibiotic resistance mechanism in biofilms to create new therapeutic approaches, more research is required. Also, studies that go greater detail into how and why these bacteria in biofilms might defend themselves from antimicrobial substances.

CONCLUSION

Microorganisms that create biofilms pose a significant threat to the medical industry. Bacteria that create biofilms are encapsulated in a matrix that protects them from human immunological reaction and antibiotics. Biofilm-forming bacteria can develop structural barriers as well as physiological modifications like slow growth and prolonged cell production. When this occurs, antibiotics are unable to stop, eliminate, or suppress the slow-growing, persistent cells present inside the biofilm matrix. As a result, chronic infections brought on by biofilms are sometimes difficult to properly treat, in part because of biofilms' resistance to antimicrobial therapy. In terms of the health industry and food safety, biofilm formation and antimicrobial resistance generally become an escalating and unsolvable challenge.

In conclusion, bacterial biofilms use a variety of strategies to resist antibiotic therapy. Depending upon the antimicrobial agent in consideration and all the circumstances surrounding the biofilm's establishment, different resistance and tolerance mechanisms have varying degrees of importance. We will go a step closer to better clinical outcomes for patients with chronic, treatment-refractory biofilm-based infections by analysing the intricate, multifactorial nature of antibiotic resistance and tolerance specific to biofilms.

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