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Review Article

Biofilm Resistance to Antimicrobial Agents and Novel Approaches to Combat Biofilm Mediated Resistance in Bacteria

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Abstract

A biofilm consists of group of homologous or heterologous bacteria enclosed in a matrix made up of protein, DNA and polysaccharide. These biofilms are tolerant to disinfectants, antibiotics, phagocytosis and defense mechanism of immune system. The survival and growth of biofilm is considered due to quorum sensing and mutations. The strategies followed to combat biofilm induced resistance are using analogues of quorum sensing molecules, metagenomics, targeting bacterial virulence factors and use of biofilm dispersing molecules. Combining ethnopharmacology with modern synthetic biology may also aid in getting rid of the antimicrobial resistance. This review focuses on the mechanism of biofilm formation, modern techniques used for combating the antimicrobial resistance, the shortcomings of pharma industry and possible options for coming out of this hurdle.

Keywords: *Biofilm; Antibiotic Resistance; Quorum Sensing; Metagenomics; Ethnopharmacology*

Abbreviations

EPS: Extracellular Polymeric Substances; AHL: Acyl-Homoserine Lactones

Introduction

A Biofilm consists of assembly of microbial cells attached to a surface and enclosed within a layer of extracellular polymeric substances (EPS) [1]. The bacterial community residing within EPS may be homogenous or heterogenous. The main constituents of EPS are polysaccharides. However, depending on the place where biofilm is present, other components like silt, blood constituents, minerals etc. may also be found. Due to the high elastic nature of these biofilms [2,3], they can withstand high mechanical stress. The nutrients and water are trapped inside these biofilms and are responsible for microbial growth within the microenvironment of biofilm. Moreover, the EPS is responsible for inhibiting the penetration of antibiotics and thus rendering antibiotic resistance to group of microorganisms enclosed in the vicinity of biofilm. In today's scenario, the antibiotic resistance due to biofilm formation poses a serious threat. This biofilm causes serious infection through medical devices which may be infections of orthopedic devices [4], prosthetic heart valves [5], intravascular catheters [6], urethral catheters [7], vocal cord prosthesis [8], contact lenses [9]. It is estimated that 65% of the bacterial infection are reported to

be caused by biofilm [6,7]. In this mini-review, we hereby discuss the mechanism of biofilm formation, biofilm antibiotic resistance and new approaches to combat the biofilm induced antibiotic resistance.

Biofilm structure and its formation

Biofilm consists of bacterial colonies of homologous or heterologous bacteria and surrounded by EPS [10-12]. The shape of colonies may vary, depending on the vicinity where it is found. The microcolonies may be rod shaped or mushroom shaped [13]. The biofilm has 10-25% cells and 79-90% EPS matrix [14]. The EPS acts as a protective barrier which protects the bacterial cells from harsh environmental conditions like UV rays and unfavourable pH conditions [15]. EPS also prevents desiccation of biofilm due to the hydrated nature. EPS of gram negative bacteria may be neutral or anionic and of gram positive bacteria cationic, depending on the nature of polysaccharide. EPS production may be affected by bacterial growth, nutrients available in the biofilm and availability of carbon [16].

The Biofilm formation occurs in five steps as follows:

Reversible attachment, irreversible attachment, maturation stage I, maturation stage II and dispersion [17,18]. The first stage involves interaction between the adsorption surface and planktonic cells. This phase may be reversible i.e. the bacterial cells may get detached to the surface for a transient period but later on may get detached. This stage may last for few minutes. The second stage involves irreversible attachment of bacteria to the surface as well as to each other. This involves formation of bacterial microcolonies and the stage may last upto 2 hours. In the third stage, there is formation of EPS. Also, there is increase in thickness of microcolonies upto 10 μm. Duration of this stage may last upto 3 days. There is further increase in thickness of bacterial microcolonies in the 4th stage. The microcolonies will grow to their maximal size upto 100 μm and his stage may last upto 6 days. In the fifth and last stage the bacterial cells develop water channels for the better access to nutrients. The micro colonies acquire shell like structure. This stage may last from nine to twelve days.

During this whole process, the bacterial cells communicate with each other by signaling molecules which help the bacterial cells to sense the bacterial cell density. This process is called quorum sensing. In gram negative bacteria signaling molecules are acyl-homoserine lactones (AHL) [19] and in gram positive bacteria are oligopeptides [20].

Biofilm resistance to antimicrobial agents

The bacterial cells present in the biofilm are very resistant (10-1000 times) to the antimicrobials in comparison to the bacterial cells present outside of biofilm [21]. There are three proposed hypothesis to explain the resistance of biofilm to the antimicrobial agents.

The first hypothesis explains that the antibiotics may not be able to penetrate inside the biofilm. This is due to the fact that the biofilm has a thick layer of EPS. The EPS forms a barrier for the penetration of extraneous substances [22]. The substances present in the EPS matrix has negative charge and this may bind to the antibiotics with positive charge, thus, reducing the diffusion across biofilm [23,24]. The second hypothesis is based on the fact that some of the bacteria may get into slower growth rate which could be due to lack of nutrients or accumulation of deleterious metabolic products [25]. Thus, this slow growth may protect the bacterial cells from the effect of antibiotics. According to third hypothesis, there is a sub-population of bacterial cells whose differentiation is common to the spore formation in bacterial cells. These cells are highly resistant to antibiotics [15].

Infections mediated through biofilm

The biofilm formed by several bacteria may infect the medical devices like catheters, heart valves, dialyzers etc. and when these devices are used for medical purposes inside human body, may cause other serious medical conditions. The bacteria colonizing these medical devices are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus* spp., *Escherichia coli, Corynebacterium*, *Clostridium* etc. Bacteria present in the biofilm forms a major impact on the persistency of infection associated with the medical devices. This leads to the

easy evasion of these pathogens into the host body and resist the antimicrobial effect of antibiotics [26,27]. Devices like contact lenses, intrauterine devices, heart valves, venous catheters, pacemakers, replacement joints and urinary catheters are prone to biofilm formation [28,29]. Initially, these medical devices may be colonised by a single species of bacteria, however, after a time period it may be colonised by multi-species [30]. The most common infection caused due to the infected medical devices is of *Staphylococcus epidermidis* and *Staphylococcus aureus*. This could be due to the fact that *Staphylococcus* are the commensals present on human skin [31]. Similarly, *Escherichia coli* is also commonly found in implanted foreign devices. It is one of the most common pathogen present in urogenital infections [32]. Another gram-negative bacteria *Pseudomonas aeruginosa* is the common agent found in lungs of patients suffering from cystic fibrosis [33].

Strategies to combat antimicrobial resistance of bacterial biofilms

Antibiotics are the compounds which are used to inhibit the growth or to kill the microorganisms. However, in present scenario, indiscriminate use of antibiotics has led to the antibiotic resistance to microorganisms [34]. The bright example is Penicillin which was the first antibiotic discovered by Alexander Fleming. However, it was found that within first seven years of its discovery about 50% of *Staphylococcus aureus* isolates were found resistant to Penicillin [35]. Moreover, 70% of pathogenic bacteria have found to be resistant to the presently used other antibiotics [36]. Thus, there is requirement of synthesis and discovery of novel antimicrobials to tackle drug resistant pathogens [37-39].

As a part of novel antibiofilm drug development, several strategies have been adopted. One of the strategies is that the bacterial colonies in biofilm get dispersed due to production of certain compounds by the bacterial cell. This is a normal part of developmental cycle and is an essential part for the biofilm dispersion [40]. It helps the bacterial colonies to expand in new habitats. Thus, development of compounds that aid in biofilm dispersion is an inquisitive method to control antibiotic resistance. This method of biofilm dispersion has been well demonstrated in case of *Bacillus subtilis*, where addition of D-amino acids led to the dispersal of biofilm [41]. This D-amino acid, as a normal course is produced by the dispersing cells of *Bacillus subtilis*. Similar observations were made in case of *Staphylococcus aureus* and *Escherichia coli* where polyamine non-spermidine is reported to disrupt the biofilm [41]. Addition of nitric oxide, which acts as a signalling molecule in many bacteria, has also been reported to result into dispersal of biofilm [42,43].

Another strategy is targeting cell signalling that is quorum sensing. This can be achieved by using compounds that have structural similarity to quorum sensing molecules. This has been observed in case of gram negative bacteria where synthetically synthesized furanones have structural similarity to AHLs and thus binds with the AHL binding site. This leads to reduction in quorum sensing and thus inhibits biofilm formation [44-46]. The drugs inhibiting biofilm formation can be used with the traditional antimicrobial drugs which acts at the cellular level. Thus the synergistic effect of both therapies can be more beneficial rather than using a single drug.

Bacterial infection can also be controlled by targeting their virulence factors, instead of targeting their viability. Since virulence factors are not essential for the survival of microbes, hence, its targeting has the least chances of developing antimicrobial resistance and also generates antimicrobials with novel mechanism of action [47]. The seven well-recognized bacterial virulence factors, such as (1) quorum sensing, (2) bacterial biofilms, (3) bacterial motility, (4) bacterial toxins, (5) bacterial pigments, (6) bacterial enzymes, and (7) bacterial surfactants. As the virulence factors are diverse in nature, the combination of antimicrobial agents with synergistic effect may be a useful tool for treatment of antimicrobial resistant bacterial infections. Out of this, the natural products form a pivot role in the pharmaceuticals development [48].

Most of the antibiotics used nowadays are derivatives of natural products, however, due to structural complexity, synthesis of these compounds is difficult [49-51]. Natural products from marine are also an alternative [52,53]. So far, the number of natural compounds available is more than 1 million [54]. Metagenomics is an alternate source for the discovery of novel compounds where the genes of microorganisms are screened directly from the environment. This technique is mainly used for microorganisms whose in-vitro culture is not possible [55,56].

However, inspite of the urgent need of effective antibiotics, very less financial input has been invested by the pharma industry for antibiotic discovery. This could be due to less expected return from the research inputs. Moreover, there are certain strict rules for antibiotic development which may hinders the rapid entry of novel antibiotic into the pharma market [57]. The average time for synthesis of a drug upto its entry into the market is approximately ten years [58]. The pharma companies are much more interested to pick up the lifestyle diseases like diabetes, obesity and heart stroke which has high money returns in the market [35,59].

Conclusion

There should be dedicated labs designed specifically for the purpose of discovery of novel antimicrobials. Discovery of novel plants and their pure compounds for their antimicrobial activity may also be an alternative. Traditionally, these plants have been used by several generations for the treatment of ailments. Therefore, in such a condition where there is paucity of a robust antimicrobial drug, combination of ethnopharmacology with modern synthetic biology may play a key role. The combine use of two medicines may be the turning point where the humanity may conquer and overpower the antimicrobial resistance of microbes.

Conflict of Interest

Authors do not have any conflict of interest.

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