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ANTIMICROBIALS RESISTANCE CHALLENGING IN INFECTIOUS DISEASE TARGETTING – A REVIEW

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ABSTRACT

The ability of microorganisms to form biofilms is closely related to infectious diseases and environmental and biotechnological processes. Biofilms constituting a microbial many cellular lifestyle and are defined as organized communities of bacteria, collaborating among themselves and being attached to an inert or living surface contained in a self-produced polymeric matrix made chiefly of exopolysaccharide. The structural nature of the biofilms and the characteristics of the sessile cells, produce resistance towards the antimicrobial agents, leading to a protected environment against adverse conditions and the host´s defenses. Despite decades of research, very little is known about the molecular mechanisms of antibiotic resistance in biofilms. Although several theories have been proposed, the precise mechanism of how this sensitivity is altered has still not been clarified. Nevertheless, it is possible to separate these mechanisms into intrinsic (or innate) and extrinsic (or induced) resistance factors to biofilms. Nevertheless, because of the different nature of biofilms, it is likely that multiple mechanisms of antimicrobial resistance occur. However, additional mechanisms must also exist to be able to account for increased biofilm antibiotic resistance. Although methods to test biofilm-growing bacteria have already been developed, their clinical relevance about the prediction of clinically successful therapies still awaits confirmation.

Keywords: Biofilms; antimicrobial agents

INTRODUCTION

The group of Dr. Costerton, in 1978, used the name "biofilms" as a more generic term for microorganisms adhering to wet surfaces in freshwater ecosystems **[1]**. Widely accepted description of biofilms done by Donald (2002) **[2]**. Bacteria exist in two principal forms, as free-floating planktonic replicating cells, and in biofilms. Microbiologists, due to historical reasons, have traditionally focused on the results of empirical research work on free-floating microorganisms growing in suspension in a liquid growth medium. However, it is now generally acknowledged that the majority of microbial cells on earth are living in spatially various communities, referred to as biofilms, a form in which they behave very diversely. It is now known that 99 % of all bacteria exist in biofilms, with only 1 percent living in the planktonic state. As it has been estimated that 65% of microbial infections are associated with biofilms **[3-4]**, this is now one of the hottest topics in microbiology **[5]**.

Microbial l multicellular lifestyle established biofilms and are termed as communities with the arrangement of bacteria, work together among themselves and being committed to an inactive or living surface contained in a self-produced polymeric matrix made principally of exopolysaccharide **[2]**. This matrix consists of many types of carbohydrates, proteins, and genes originating from microbes, and the bacterial consortium contains one or more species living in a sociomicrobiological way. Direct structural examination of biofilms has shown that their component micro-colonies, which are composed of cells embedded in matrix material, are bisected by ramifying the water channels that carry the bulk fluid into the community by convective flow **[6, 7]**. This allows cells to form long-term relationships, interact with each other and establish metabolic cooperation. Biofilms can be composed of a population developed from a single species or a community-derived of multiple microbial species. Hypotheses about the ecological advantages of forming biofilms include protection from the environment, nutrient availability, metabolic cooperation, and the acquisition of new genetic traits **[8]**.

Microbial multicellular communities or biofilms are of diverse sizes and shapes, with most common types containing mushroom-like, pillar-like, hilly, or flat many cellular structures, which allow cells to form long-standing relationships, interact with each other and start metabolic support **[8, 9]**. The bacteria interaction with a surface and the

development of a biofilm can be seen as a survival mechanism, with bacteria acquiring nutrients and protection from biocides acts as a useful mechanism. In cases of adverse conditions such as desiccation, osmotic shock, or exposure to toxic compounds, UV radiation, or predators, the microbial community can provide protection. Moreover, biofilms are also sites where genetic material is easily exchanged because of the proximity of the cells, thus maintaining a large gene pool.

Stages of biofilm development

Adhesion of pioneer bacteria is the initiation step by some of the planktonic or free-floating bacteria and they move towards the surface (live or alive) and attached to the "boundary layer" which is the inactive zone at the surface where the flow velocity falls to zero. Some of these cells strike and are adsorbed to the surface for only a finite time, before being de-adsorbed, in a process called "reversible adsorption" **[10]**. This initial attachment is based on electrostatic attraction and physical forces, but due to not any chemical attachments. Some of these reversibly adsorbed cells begin to prepare for a lengthy stay by forming structures which may then permanently bind then to the surface within the next few hours, the pioneer cells proceed to reproduce and the daughter cells, which form microcolonies on the surface and begin to produce a polymer matrix around the microcolonies **[8; 11]**, in an irreversible steps. Subsequently, in the next stage focal areas of the biofilm dissolve and the liberated bacterial cells are then able to spread to other locations where new biofilms can be formed, and the mature biofilm may contain water-filled channels and thereby resemble primitive, multicellular organisms and the attachment is mediated by extracellular polymers that extend outward from the bacterial cell wall. This polymeric material, possess charged and neutral polysaccharides groups that not only provide attachment but also act as an ion-exchange system for concentrating trace nutrients from the overlying water and for tricking.

Biofilms are permeated at all levels by a network of channels through which water, bacterial garbage, nutrients, enzymes, metabolites and oxygen move to and fro, with gradients of chemicals and ions between microzones providing the power to shunt the substances around the biofilms **[12]** Oxygen may be depleted within only 30-40 □m of the water/biofilm interface. Although the precise depth of the oxygen gradient in the biofilm varies depending on the oxygen content in the bulk water, water temperature, and water flow, this gives a rough idea of

how far oxygen can diffuse. Some general features of biofilm infections in humans compared with acute planktonic infections are shown below **[13]**:

1. Aggregates of bacteria embedded in a self-produced polymer matrix
2. Tolerant to both innate and adaptive immune responses
3. Tolerant to clinically dosing of antibiotics despite susceptibility of planktonic cells
4. Chronic infections

Biofilms and protection from antibiotics

In the traditional antibiotic resistance of planktonic bacteria, usually involves inactivation of the antibiotic, modification of targets, and exclusion of the antibiotic **[14]**. These actions typically require the acquisition of specific genetic factors, such as genes for beta-lactamase or efflux pumps.

Bacterial biofilm formation crucial feature is the resistance improvement of the integral microbes to antibiotics and other stressors, The characteristics of the sessile cells, produce resistance towards the antimicrobial agents, leading to a protected environment against adverse conditions and the host´s defenses is due to the structural arrangement of these films **[15]**.

Innate resistance factors to biofilms

The intrinsic factors of resistance are activated as part of the biofilm developmental pathway, which are integral parts of the biofilm structure and physiology resulting from conversion to a biofilm lifestyle. Factors affecting antibiotic resistance have been identified and it is due to several different intrinsic biofilms. For example: matrix of these biofilms might act as a diffusion barrier; small and specialized environment of biofilms can be established inside; some kinds of bacteria within the biofilms may differentiate into persisters which are also within the bacterial population; an increased production of oxidative stress causes might change in the physiology of bacteria; and an antagonist of antibiotics and degradation mechanisms may be active in some parts of the biofilms **[16, 17]**.

Diffusion barrier

Biofilms to prevent antibiotics from reaching their goals can act as bodily diffusion barriers. Antibiotic was shown to be able to penetrate these structures through a thick mixture of exopolysaccharide, DNA, and protein to reach the targets **[18]**. However, this mixture was not able to achieve an effective concentration in some all parts due to the physical and/or chemical properties of the matrix, which resulted in an apparent increase in resistance. However, limited antibiotic diffusion does not appear to be a

universal trait shared by all biofilms, and, there is conflicting data about whether the biofilm matrix is a major contributing factor influencing biofilm resistance. A decreased penetration and diffusion of antimicrobials through the biofilm matrix has been shown to influence biofilm survival in some cases. For example, at sub-MIC concentrations of beta-lactam antibiotics, an increased alginate synthesis in P. aeruginosa biofilms was induced and enhancement of the biofilm matrix of some slime-producing coagulase-negative staphylococci.

Microenvironments within biofilms

The nutrients and oxygen inside biofilms reduction might cause an altered metabolic activity and developed slow growth of the bacteria. Several studies have revealed oxygen limitation and the presence of hypoxic zones deep within biofilms, with nutrient diffusion through biofilms being restricted **[19]**. Inspection of environmental as well as in vitro biofilms has revealed that the oxygen concentration may be high at the surface, but low in the center of the biofilm where anaerobic conditions may be present. Likewise, growth, protein synthesis, and metabolic activity are stratified in biofilms, for example, there is a high level of activity at the surface but a low level in the center, resulting in slow or no growth. This fact is one of the explanations put forward for the reduced susceptibility of biofilms to antibiotics **[20]**.

Differentiation into persister cells

Persisted cells are either no growing or slow-growing and have a greatly reduced susceptibility to antibiotics **[21]**. In the persister's theory, these small subpopulations of bacteria can survive extreme antibiotic treatment and have been assumed to be the product of phenotypic differences rather than due to stable genetic changes these can be found within the biofilms differentiate into dormant cells.

Increased production of oxidative stress

Oxidative stress is caused by an imbalance between the production of oxidants, such as the free radicals, peroxide and nitric oxide, with the levels of antioxidant defenses. A disturbance in the prooxidant-antioxidant balance in favor of the overproduction of reactive oxygen species (ROS) can result in damage to the cellular components, including the matrix, DNA, proteins, and lipids **[22, 23]**. Diverse stresses, including nutrient availability, low oxygen, high osmolarity, ethanol, and sub-inhibitory antibiotic concentrations, can alter the cellular functions associated with the oxidative metabolism **[24]**, thereby stimulating the production of ROS and the highly reactive

hydroxyl radicals (HO.), which are generated by the presence of hydrogen peroxide (H2O2) and iron (Fenton reaction) either by the superoxide anion (O2 -) or by the superoxide anion, hydrogen peroxide and a metal catalyst (Haber–Weiss reaction) **[25]** . In the antioxidant defense system, the main enzymes involved in the detoxification of ROS are superoxide dismutase (SOD) and catalase (CAT), among others **[26]**. However, in oxidative imbalance with due to an overproduction of ROS, a reduction in the oxidative defenses is insufficient to remove the free radicals, and therefore, the antioxidant system plays a very important role in the control of this process **[27]**. The increased production of oxidative stress causes changes in the physiology of bacteria, with specific phenotypic alterations occurring, and we have observed that biofilm development is influenced by the balance between the production of oxidants (ROS and NO) and the levels of antioxidant defenses (SOD), which can be significantly affected by different environmental stresses.

Antagonist action of antibiotics and degradation mechanisms active in some parts of biofilms

Microenvironments exits that can antagonize the action of antibiotics, and the degradation mechanisms active in some parts of biofilms may also be involved. Sociomicrobiology is defined as the relation existing between quorum sensing (QS) and biofilms. Bacteria communicate using synthesizing and reacting on signal molecules to sense when a concentration of bacteria is present in a limited space in the environment and then respond by activating certain genes that produce, for example, virulence factors such as enzymes or toxins. The most well-described QS molecules in Gram-negative bacteria are the N-acyl-l-homoserine lactones, and in many Gram-positive bacteria, the QS molecules are small peptides. QS can regulate the production of virulence factors such as extracellular enzymes and cellular lysins, which are important for the pathogenesis of infections, where the bacteria functions as a protective shield against phagocytes **[28]**. QS may also influence the development of the biofilm and determine the tolerance of biofilms to antibiotic therapy and the innate inflammatory response **[29]**.

CONCLUSION

During their evolution, bacteria have been able to develop successful strategies for survival, which include an attachment to surfaces and the development of protective biofilms where bacteria behave very differently to the free-floating types. These

successful strategies make it difficult to control biofilm growth, with a biofilm providing bacteria with a 10- to 1,000-fold increase in antibiotic resistance compared to free ones. Due to the heterogeneous nature of biofilms, it is likely that multiple mechanisms of antimicrobial resistance are useful to explain biofilm survival in several cases, the result of an intricate mixture of intrinsic and extrinsic factors due to antibiotic resistance.

Much more research is needed to reveal additional and/or novel antibiotic-induced factors in biofilms, as the multifactorial nature of biofilm antibiotic resistance has hindered the identification of these pathways, with much still being unfamiliar about the induced factors in biofilm resistance. Discovery of these factors should lead to new and better treatments for biofilm-related infections.

Novel approaches are vital to overcoming biofilm antibiotic resistance by through the development of innovative therapies aimed at killing the constituent bacteria & disrupting biofilms, with the management of intrinsic and extrinsic resistance pathways providing much promise for future treatment of biofilm infections.

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