The Role of Bacterial Biofilm in Enhancing Virulence and Therapeutic Resistance: A Comprehensive Review

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Abstract— The issue of bacterial biofilms may be deemed one of the significant problems of modern medicine and microbiology. These surface bound microbial communities of high density are enclosed by an extracellular polymeric substance (EPS) matrix that is self-secreted to shield them against antimicrobial agents and immune systems of the host. Approximately 80 percent of all microbial infections in human beings are associated with biofilm infection resulting in chronic and recurring illnesses that are notoriously difficult to treat. This is a review that detailed the study on the role of biofilms in enhancing the virulence of bacteria and therapeutic resistance. We discuss the stages of biofilm formation, the organization, and purpose of EPS, the quorum sensing in the behavior coordination of the bacterial cells and the persister cell formation. In addition, we explore the numerous resistance mechanisms employed by the biofilm entrenched bacteria as the reduced penetration of the antibiotics, regulated micro environment, horizontal gene transfer, and phenotypic heterogeneity. These mechanisms are also studied, which is significant in the development of new treatment measures to counter biofilm-related diseases.

Keywords- Bacterial biofilm, antimicrobial resistance, virulence factors, quorum sensing, extracellular polymeric substances, persister cells, chronic infections.

I. INTRODUCTION

Bacterial biofilm is an organised aggregate of microorganisms that stick to the walls of biotic or abiotic surfaces and are found in an autogenic manufacturing of an extracellular polymeric constitution (EPS). It is the most widespread type of growth and most bacteria in the natural environment are not present as planktonic cells in biofilm, but rather as part of biofilm. The shift of biofilm to planktonic and vice versa is a radical shift in bacterial physiology, gene expression, and behaviour that has extended consequences on human health, industrial processes, as well as environmental systems [1].

The biofilm-related infections constitute an important clinical problem and approximately 80 percent of all bacterial infections in humans. These include the chronic wound infections, the catheter related urinary tract infections, the prosthetic joint infections, the dental plaque, the chronic lung infection in patients of cystic fibrosis and the medical implants and devices infections [2]. The clinical significance of biofilms can be explained by the fact that biofilm-related bacteria

are far more critical to antimicrobial therapy, the antimicrobial resistance of biofilm-related bacteria to a planktonic bacterium is 10 to 1,000 times higher [3].

A number of mechanisms are interrelated in explaining the increased virulence and resistance to treatment which is observed in biofilms. The EPS matrix provides a physical barrier, which inhibits penetration of antibiotics and protects the bacteria against immune cells of the host. Moreover, biofilm microenvironmental heterogeneity facilitates the growth of antibiotic tolerant persister cells. The horizontal gene transfer of the virulence factors and the co-ordinated expression of the virulence factors is helped by the quorum sensing systems. This review shall attempt to provide a comprehensive review of these mechanisms and how they implicate to clinical management of biofilm-associated infections.

II. BIOFILM FORMation and structure BIOFILM formation and structure

The formation is characterized by an outer layer of biomass which is fixed on the substrate surface by using aspartame formidop, arbiterin, and glucose (Shahid, Sadehi, and Hajizadeh, 2019).

Biofilm formation is a dynamic process that can be categorized into five stages (1) reversible binding, (2) irreversible binding, (3) production of EPS and microcolony, (4) biofilm maturation and (5) dispersal [4]. Multifaceted signaling systems and environmental signals control all the stages and aid in the adaptation of bacteria to the changing environment.

A. Initial Attachment

The initial attachment of the planktonic bacteria to a surface is done through the weak reversible forces of van der Waals forces, electrostatic forces, and hydrophobic forces. At this phase, bacteria can still dissociate themselves, and return to the planktonic state. Hydrophobicity, charge and roughness are surface properties that are important in the first attachment process. Some of the host matrix proteins applicable in the clinical setting include fibronectin, fibrinogen and collagen; these coats implanted medical devices rapidly availing the bacterial adhesins additional sites of bonding [5].

B. Irreversible Attachment Production of EPS

In cases where irreversible attachment is realized, bacterial adhesins, including pili and fimbriae, and cell surface proteins, having stronger bonds to the substratum are produced. Meanwhile, the bacteria begin to produce EPS ingredients, which solidify the bond and cell-cell attachment. Cyclic-di-GMP (c-di-GMP) is the mediating molecule in this switch and higher intracellular levels are facilitating the switch between motile and sessile lifestyle and are triggering the EPS production [6].

C. Biofilm Maturation: Architecture 3D.

The biofilm develops a classic three-dimensional shape and the biofilm further increases in size and develops mushroom-like or tower-like formations between which waterways flow transporting nutrients and eliminating wastes. This arrangement of the structure generates various micro environments comprising of various oxygen levels, pH levels and nutrient levels. The cells of outer layers are typically actively metabolizing and growing extremely fast, and the cells of inner layers are nutrient-limited and enter a dormant state [7]. This heterogeneity is inherent as far as resistance of biofilms to antimicrobial therapy.

III. EXtracellular Polymeric Substance Matrix.

The EPS matrix comprises 50-90 percent of the total organic matter in biofilms and is the feature that defines the difference between the biofilm communities and the planktons population. It is a gel-like structure composed of polysaccharides, proteins, external DNA (eDNA) and lipids and the precise composition of this varies with the bacterial species, environmental conditions and age of biofilm formation [8].

A. Polysaccharides

Exopolysaccharides are the most crucial structural components of EPS matrix. Pseudomonas aeruginosa has three types of polysaccharides namely alginate, Pel and Psl. Alginate is an anionic polymer made of mannuronic and guluronic acids which is particularly applied in the treatment of the chronic lung infection in cystic fibrosis patients and which also makes itself a contributor to the mucoid phenotype. The complementary roles of Pel and Psl polysaccharides in biofilm structure and anti-biotic resistance. Similarly, Staphylococcus aureus produces poly-N-acetylglucosamine (PNAG) that is required in the synthesis of intercellular adhesion and biofilm integrity [9].

B. Extracellular DNA (eDNA)

Active secretion release and cell lysis releases eDNA which also has diverse roles in biofilms such as the support of the structure, initial attachment, genetic exchange via horizontal gene transfer and also a source of nutrient when starved. eDNA is also present which also implicates in antibiotic resistance as it induces the sequestration of cationic antibiotics, antimicrobial peptide due to electrostatic interaction [10]. It has been shown that the structural role of eDNA is available since the DNase treatment has been proven to prevent the creation of biofilms and to sensitize the various species to the antibiotics.

This is brought about by the presence of protein and enzymes in the egg. Matrix Proteins and Enzymes: This is because protein and enzymes are found in the egg.

The components of the protein component of EPS matrix are the structural protein, enzymes and amyloid fibers. They aid in the stability of the matrix these proteins enable them to adhere to surfaces, host tissues as well as provide enzyme activities which can destroy host immune factors and antibiotics. The amyloid fibres such as curli in Escherichia coli, and phenol-soluble modulins in S. aureus provide extraordinary high mechanical strength of the biofilm structure. The matrix-associated enzymes such as 2-lactamases also have the capability of inactivating the antibiotics before they can get to the target bacteria [11].

IV. VIRULence regulation and quorum Sensing.

The cell-cell interaction system is known as Quorum sensing (QS) through which bacteria can control gene expression according to population density. It is a mechanism that is achieved by the manufacture, discharging, and sense of signaling molecules known as autoinducers. When the autoinducer concentrations get to a certain threshold, which correlates with a critical cell density, specific receptors are activated and therefore, an organized change in gene expression that affects biofilm development, the production of virulence factors and antibiotic resistance ensues [12].

The Gram-Negative Bacteria A. Quorum Sensing Systems.

Autoinducers N-acyl homoserine lactones (AHLs) are predominantly used by gram-negative bacteria in their QS. Las and Rhl systems: The systems are hierarchical systems of regulation that control the production of numerous virulence factors including elastase, rhamnolipids, pyocyanin

and hydrogen cyanide in P. aeruginosa. On the top of this hierarchy is Las system which generates and reacts to 3-oxo-C12-HSL and positively influences the Rhl system. It is demonstrated that the absence of QS components causes the decrease of the virulence of P. aeruginosa by several folds proving the unquestionable importance of such systems in the pathogenesis [13].

G + C: The Gram-positives are Gram-positive bacteria. The Gram-Positive bacteria Quorum Sensing.

In gram-positive bacteria, gram-positive bacteria use autoinducing peptides (AIPs) as the major QS signals. Accessory gene regulator (agr) system S. aureus produces cyclic thiolactone peptides that are used to regulate the expression of surface proteins, exotoxins and enzymes to kill tissues. Agri system: In order to controll the adhesins down-regulation and secreted toxin and proteases up-regulation, the agr system is initiated upon agr system alteration to colonization to invasion. Interestingly, different strains of S. aureus produce different versions of AIP that leads to intergroup competition and interference [14].

C. Autoinducer-2 and Inter-Specific Communication.

Autoinducer-2 (AI-2) is a general signal transducer that is a furanosyl borate diester and enables cross-bacterial communication between polymicrobial biofilms. The luxS gene which encode the production of AI-2 are general in a large range of Gram-negative and Gram-positive groupings. There is the possibility of cross-species, which incorporates AI-2 signaling in polymicrobial infections, increasing the overall community virulence and antibiotic resistance. It is specifically in more complicated clinical situations such as chronic wound infections and oral biofilms where the interspecies communication can be applied [15].

V. AntiBiotic Resistance in BIOfilms.

Extraordinary antibiotic resistance of bacteria in biofilms is multifactorial in nature which is defined by physical, physiological, and genetic processes which interact to protect the bacterial population. Unlike the genetic-mediated resistance in the traditional ways, the resistance due to biofilms is primarily a phenotypic resistance which is reversible upon spreading to the planktonic state [16].

A. Resistant penetration of Antibiotics.

The EPS matrix acts as a diffusion barrier that could not allow drugs to enter the inner layers of the biofilm with antibiotics. The permeation of the antibiotics through the matrix will depend on the molecular properties of the antibiotic, the composition of the EPS and the electrostatic interactions. Positively charged aminoglycosides can be taken up by the negatively charged components of EPS, in particular, alginate and eDNA. In addition, the antibiotics can either be degraded enzymatically or destroyed chemically since they slowly enter the matrix to attack cells at subinhibitory levels [17].

B. Microenvironments Alteration.

The microheterogeneous nature of biofilm provides the conditions with potential widespread implications on antibiotic activity. The metabolism of the active cells on the surface results in the development of oxygen gradient resulting in the development of the hypoxy or anaerobic environment of the deeper layers. The formation of S. aureus biofilms is 21 fold in hypoxic condition, and this is connected to the resistance to neutrophil phagocytes. Also, local pH changes and accumulation of waste products may result due to metabolic activities and may affect the

activity of pH-sensitive antibiotic agents, such as amino-glycosides, the bactericidal action of which is inhibited in acidic environments [18].

Cells D C persister Cells and Dormancy.

Persister cells are a subpopulation of phenotypically tolerant cells which can survive in conditions of high concentrations of antibiotics, and do not necessarily carry resistance genes. These cells enter a dormant state which is characterized by a highly lowered rate of metabolism, arrested growth and a reduced activity of biosynthetic pathways. It is possible that dormant persisters are able to evade the affects of antibiotics by silencing these targets owing to the fact that most bactericidal antibiotics are designed to prevent an active cell process e.g. cell wall synthesis, protein synthesis or DNA replication. The biofilms are enriched with persister cells (not exceeding 1 percent of the populations at the stationary phases) contributing to the chronic and recurrent infections [19].

The development of persister cells is controlled by toxin-antitoxin (TA) systems, and stress responses, and stringent response. The toxins (RelE, MazF, and HipA) are overexpressed to cause the development of multidrug resistance by suppressing key functions such as translation. When antibiotic pressure is relieved, persisters have a chance to revive and recolonize the site of infection, which results in a relapse. The phenomenon underlines the recurrent nature of most biofilm-associated infections even after the supposedly effective treatment with antibiotics [20].

D. Horizontal Gene Transfer

The eDNA contained in the matrix and the proximity of the cells in biofilms provide the best environment to induce horizontal gene transfer (HGT) of antibiotic resistance determinants. The high concentrations of eDNA in the matrix are important in facilitating transformation with free DNA and in conjugating in biofilms, as compared to planktonic cultures. The additional method of HGT is outer membrane vesicles (OMVs) that are released by Gram-negative bacteria, and biofilm-derived OMVs have been demonstrated to be more efficient in facilitating the transformation of planktonic populations [21].

The stress caused by the antibiotic also triggers the SOS response that also causes the further promotion of HGT as it results in the expression of integrases, transposases, and other mobilization factors. Notably, aminoglycoside resistance genes, b-lactam resistance genes (including extended-spectrum b-lactamases and carbapenemases) and other antibiotic-resistance genes may be effectively spread across biofilm communities, which may make the rapid dissemination of multidrug resistance [22].

VI. VIRULence MEchanisms VIRulence Mechanisms related to BIOFILM.

In addition to making bacteria resistant to antibiotics, biofilm formation increases bacterial virulence by promoting colonization, tissue destruction, and immune evasion in a variety of different ways. The biofilm way of life allows the pathogens to develop persistent infections despite the impact of antimicrobial treatment and the host immune responses [23].

A. Increased Colonization and Persistence

The formation of biofilms allows bacteria to form a safe shelter wherein they are able to be sustained over long periods of time. The EPS matrix preserves the mechanical removal, desiccation, and environmental stresses of the bacteria. Biofilms on clinical equipment in the clinical practice act as reservoirs where bacteria are able to continuously seed the tissue or blood

around the equipment, resulting in recurring or disseminated infections. Capability to colonize biofilms on a host tissue is also what allows the chronic colonization, e.g., cardiac valves in endocarditis or pulmonary epithelium in cystic fibrosis [24].

B. Host Immune Response Evasion of Host Immune Response.

The EPS matrix gives a physical protection against neutrophil and macrophage phagocytosis. The immune cells find it difficult to kill the bacteria in a biofilm, even when they are capable of penetrating the outer layers of the biofilm. Moreover, hypoxia in biofilms inhibits the respiratory burst of neutrophils that limits their bactericidal ability. Bacteria immobilized in biofilms also produce virulence elements that actively inhibit the immune cell activity, which encompasses proteins that break down immunoglobulins and complement factors, and toxins leading to the death of immune cells [25].

C. Co-ordinated Virginity Factor Expression.

Quorum sensing facilitates the co-ordination of the expression of virulence factors at population levels that are high enough to overwhelm host defense. In P. aeruginosa, QS regulates the expression of the acute infection-essential factors of elastase, alkaline protease, exotoxin A, and the type III secretion system effectors. Having the biofilm form of growth enables the bacteria to attain the critical cell concentrations necessary to trigger QS and sheltering them against immune clearance. This regulation makes sure virulence factors are expressed in the best time and quantity to damage tissues and enhance the spread of the bacteria [26].

VII. Therapeutic Interventions to Biofilm.

The shortcomings on the use of conventional antibiotic therapy against biofilms have necessitated development of new therapeutic interventions. The present methods are aimed at the prevention of biofilms formation, interference with the biofilm formation, or improving the strength of current antibiotics against biofilm-related bacteria [27].

A. Quorum Sensing Inhibitors

Quorum sensing inhibitors (QSIs) have been put forward as a potentially effective form of anti-virulence technology, which focuses directly on bacterial communication, and may lower the intensity of selective pressure on the development of resistance. The QSIs disrupt the synthesis of autoinducers, signal transmission, or receptor attachment, which inhibit the group expression of virulence factors and biofilm formation. QS inhibitory activity has been shown by natural products, including furanones and AHL analogs as well as plant-derived products, like quercetin and garlic extract. Research has revealed that QSIs have the potential of enhancing the vulnerability of bacterial biofilms to antibiotics in vitro and in vivo [28].

B. EPS-Targeting Approaches

Alteration of the EPS matrix has the potential to increase the penetration of antibiotics and exposing bacteria to immune effector pathways. The matrix can be destabilized by enzymatic degradation in the presence of DNase I, dispersin B, or alginate lyase as well as induce the dispersal of biofilm. Approaches mediated by nanoparticles have demonstrated some specific promise with silver nanoparticles reducing MRSA and MRSE biofilm formation by more than 95% by direct EPS disruption. The positively charged nanoparticles are attracted to the negatively charged EPS matrix and the drug is delivered to the bacterial target [29].

C. Bacteriophage Therapy

Bacteriophages have a number of benefits to treating biofilm infections among them include high specificity, capacity to replicate at the location of infection, and synthesis of depolymerase enzymes capable of degrading EPS components. A combination of several phages into phage cocktails is able to provide a wider host range and minimize the chance of resistance emergence. Combination of phages with antibiotics has shown synergy, which has been shown to improve bacterial killing and biofilm destruction. Nevertheless, several limitations such as limited host range, possibility of developing resistance and regulatory issues have yet to be overcome [30].

D. Antimicrobial Peptides

Antimicrobial peptides AMPs are natural molecules with a broad spectrum of activity. Their cationic amphipathic nature allows them to interact with negatively charged bacterial membrane which leads to the disruption of the membrane. Anti- biofilm effects by AMPs like LL-37 encompass inhibition of cell adhesion, disruption of biofilm promoting genes, and decrease in biofilm thickness. The fact that AMPs can be applied to both growing and dormant cells renders them quite appealing when it comes to the management of biofilm infections [31].

E. Cells that are Strategy-directed against Persister Cells.

The eradication of persister cells must involve methods that reinstate the dormant cells to antibiotics or eliminate the dormant cells. The restoration of antibiotic susceptibility can be achieved by activating bactericidal drugs targets using a process of metabolite-based strategies that induce cellular metabolism. Acyldepsipetide antibiotics (ADEPs) are a new methodology that induces the ClpP protease inducing death of growing and dormant cells through lethal protein degradation. ADP4 has been reported to be effective in combination with conventional antibiotics in animals with 100 percent eradication of S. aureus biofilms [32].

VIII. CONCLUSION

The presence of bacterial biofilms poses a serious threat to the contemporary medicine, being not only more virulent, but also with excellent resistance to antimicrobial treatment and immune reactions of the host. The interaction between the EPS matrix and the quorum sensing regulation, with persister and horizontal gene transfer, provides many layers of protection causing conventional antibiotic treatment to be ineffective to a great extent. The molecular principles of these mechanisms are crucial in the establishment of new therapeutic solutions that could surmount biofilm-related resistance.

The development of new strategies aimed at quorum sensing, EPS disruption, phage therapy, and elimination of persistor cells bring an opportunity to better control biofilm related infections. Multi-mechanism combination therapies could be the most effective. Further studies on biofilm biology and creation of biofilm-specific diagnostics and treatment are still priorities to deal with this serious problem of public health. We expect to come up with a deeper and better method against these recalcitrant infections as we continue to gain deeper insights into biofilm physiology.

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