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Biofilm: An Important Bacterial Feature Still to Deal With

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Abstract

Currently, the bacterial infections are one of the main causes of death worldwide and a public health problem for most governments. In this scenario, virulence factors are of importance, including the capacity of forming biofilm. The ability of producing biofilms is directly involved in bacterial pathogenicity and hugely worse the risk of death due to a bacterial infection. This feature allows the bacteria to colonize different surfaces (e.g. Medical devices), and causes several complications, especially in nosocomial infections. This work reviewed biolfim producing mechanisms, the relation with resistance process and the problems associated to biofilms-affected-medical devices.

Keywords: Bacterial infection, Biofilm, Pathogenicity, Resistance, Medical devices, Persisters.

Contents

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1. Introduction

The current infections represent a major public health problem with high morbidity and mortality rates [1, 2]. Recent data from the World Health Organization (WHO) and Global Burden of Disease (GBG) indicate that bacterial infections are present among the top five causes of death worldwide [3] and according to some authors will be still present in 2030 globally (Table 1). Currently, among these causes are lower respiratory tract infections and diarrheal diseases. According to the literature, bacterial infections cost 5 billion to 10 billion dollars a year in health care [4].

Table-1. The top five causes of death worldwide, according to the World Health Organization in 2015 and predictions for 2030.

Rank in 2015	Cause	Deaths (%)	Predicted Deaths in 2030 (%)*
1º	Ischemic Heart Disease	13.2	13.4 (Rank 1º)
2º	Stroke/ Cerebrovascular Disease	11.7	10.6 (Rank 2º)
3º	Lower Respiratory Infections	5.6	3.5 (Rank 5º)
4º	Chronic Obstructive Pulmonary Disease	5.6	7.8 (Rank 4º)
5º	Diarrheal Diseases	3.2	not cited on the list for 2030

Source: adapted from World Health Organization [3] and *Mathers and Loncar [5].

The ability of a pathogen to overcome the human body defenses, colonizes it and expresses pathogenicity leads to physiological changes and the development of pathological conditions [6]. Some bacteria have intrinsic characteristics, including the presence of the capsule, lipopolysaccharides, and the ability to form biofilms, which are responsible for their high pathogenicity [7]. Biofilms are involved in most of severe and chronic hospital bacterial infections with an important role in pathogenicity and resistance features. This mechanism ensures the bacterial adhesion on biotic and abiotic surfaces also protecting the bacteria from antibiotic effects and host defenses [8, 9].

Several microorganisms produce biofilms, such as fungi, protozoa and viruses. However, bacteria are predominant, especially the genus *Alcaligenes*, *Bacillus*, *Enterobacter*, *Flavobacterium*, *Pseudomonas* and *Staphylococcus*. Due to the small size, the short reproductive cycle, the capacity to adapt to adverse conditions and the production of protective extracellular substances, bacteria are among the best biofilm producers [10].

Pseudomonas aeruginosa is among the biofilm-producing Gram-negative bacteria of medical importance. This bacterium can cause sepsis, acute and chronic infections and is also involved in infections related to the insertion of medical devices such as catheters [11]. Another bacterium of medical importance is *Staphylococcus aureus*, a gram-positive microorganism involved in nosocomial infections, chronic osteomyelitis, chronic rhinosinusitis and infections associated with orthopedic implants. According to the literature, 80-90% of hospital biofilms-related infections involve this species [12]. *S. aureus* is part of the human skin microbiota and becomes opportunistic in host immunosuppression situations, when it expresses its pathogenicity [13]. This species may colonize the surface of different medical devices such as implants, catheters, syringes, prostheses and artificial valves [14].

Currently, avoiding infections associated with medical devices is of great importance. Therefore, researches targeting the development of new antibiotics that affect bacterial biofilms are in current need. These new drugs may be important not only to act directly on the biofilm but also in a prophylactic manner if used as a pre-treatment of medical devices [15]. In order to offer, especially for drug design research groups, a brief overview of this subject, we briefly reviewed different aspects related to biofilms, including its formation, its relation to the bacterial resistance and the problems involved in the infection caused by biofilm-producing bacteria.

2. Bacterial Resistance: A Really Big Issue to Take Seriously

According to several studies, including those from the Infectious Diseases Society of America (IDSA), after the year 1983 there was a substantial reduction in the number of new antibiotics released on the market [16, 17]. This lack of investment involves directly the pharmaceutical companies that prefer to invest in medications for chronic diseases rather than drugs to treat diseases that may be quickly cured. Therefore, currently it has been discussed the creation of financial incentives for developing new antimicrobial agents against multiresistant bacteria strains [18].

Resistance to antibiotics is a global health issue that involves multiple pathogens and factors [19]. According to the literature, the relationship between the bacterial resistance and some factors such as the indiscriminate use of antibiotics is still difficult to estimate precisely. In addition, some aspects have worsened this scenario worldwide such as the different countries legislation regarding the antibiotics use and availability. This is especially of concern in some developing countries where antibiotics are still available for the population use without any prescription request [20].

Food trade and the increasing movement of people globally help spreading resistant bacteria strains, making even more difficult to prevent and control this situation [21]. Currently it is about knowledge that antimicrobial resistance is an inevitable expression of the bacterial genetic evolutionary behavior, being intrinsic/natural or acquired/stimulated by external factors (e.g. Overuse of antibiotics with subsequent selective pressure) [16].

Lately, literature reported the important role of intensive care units in the emergence of the so called superbugs (multiple resistant strains) and the spread of almost intractable antimicrobial resistance. The most vulnerable patients are those hospitalized in critical sectors, such as intensive care units, neonatal nursery, transplant and dialysis rooms. The acquisition and dissemination of these microorganisms may occur through these environments by the reuse of materials and poor biosafety procedures [22].

There are two mechanisms for spreading resistance: a) the vertical transfer from a parent bacterium to another bacterium, called intrinsic resistance. Thus, an organism can be resistant to a particular class of antibiotics because of their original gene. For example, the genus *Streptococcus* is intrinsically resistant to aminoglycosides while gram-negative bacteria are naturally resistant to vancomycin [23] b) the vertically and horizontally transference, which may occur even in bacteria from different genera so called acquired resistance. It may occur by two mechanisms, including mutation in bacterial DNA or acquisition of new genetic material. Bacterial mutations occur at a frequency

of 1 in 1010 cells, whereas antimicrobial resistance genes can be transferred through plasmids, transposons and integrons that can carry more than one resistance gene [24, 25].

3. Mechanisms of Resistance: Old Aspects of a Difficult Dealing Issue

The bacteria develop resistance against antibiotics through several biochemical mechanisms (Figure 1). These mechanisms can coexist in the same bacterial strain with cross resistance to different antibiotics [26] and include:

a) Structure modification of the antibiotic by specific enzymes;
b) Modification of the antibiotic target binding site;
c) Decrease of bacterium cell permeability to antibiotics;
d) Increased synthesis of molecule/substrate that competes with the antibiotic;
e) Efflux of the antibiotic.

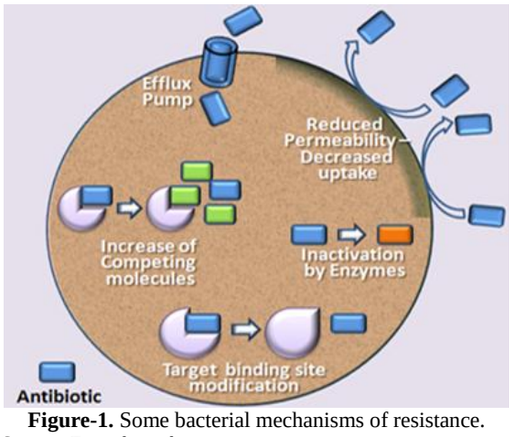

Figure-1. Some bacterial mechanisms of resistance.
Source: From the authors.

One of the most common resistance mechanism is the bacterial synthesis of enzymes that chemically modify the antibiotic. An example is the β-lactamases family, which hydrolyzes the β - lactam ring of the penicillins, cephalosporins and carbapenems [27]. Another important group of enzymes is the acetyltransferases that inactivate antibiotics through an acetylation mechanism and that are responsible for the bacterial chloramphenicol resistance [28].

The other important resistance mechanism is the modification of the target binding site since antibiotics bind to a specific bacterial site. Thus, the chemical modification of the target structure may avoid the antibiotic binding. β-lactam antibiotics bind to penicillin binding proteins [20] however, the Methicillin Resistant *S. aureus* (MRSA) strain has the SCCmec (Staphylococcal Cassette Chromosome) and the *mecA* gene that encodes the mutation of the penicillin binding protein, avoiding binding to β-lactams antibiotics [29]. Therefore, MRSA is resistant to all penicillins, cephalosporins and carbapenems [30]. Another example is the fluoroquinolones resistance, which occurs through mutations on topoisomerases that are essential for DNA replication, and the methylation of the ribosome [31].

Another bacterial strategy to avoid the antibiotic effects is to prevent the antibiotic access to its targets. Since it must reach the bacterial target site in an appropriate concentration, the increase of the permeability barrier or the presence of an efflux pump are effective scape mechanisms (Figure 1) [32]. Bacterial membrane efflux pumps are found in both Gram-positive and Gram-negative organisms pumping the antibiotics out of the cell [33]. Gram-negative bacteria may regulate the permeability of the outer membrane by expression of porins, which are membrane protein channels for passive diffusion. *P. aeruginosa* is a bacterium that has at least four efflux pumps [34].

Another mechanism of resistance is the synthesis proteins that may protect the ribosomes against the antibiotic action. These antibiotics inhibit protein synthesis on bacterial ribosomes and act against 30S subunits (Tc and GAs) or 50S (CAP and macrolides) [35].

4. Biofilm and Bacteria

In 1978, William J. Costerton proposed the name biofilm to describe the bacterial clusters found colonizing surfaces [36]. Since then, biofilms have been associated with many health problems such as endocarditis, otitis media, periodontitis and prostatitis [37]. Currently, it is recognized as a major problem in health care and food industry, and are associated with approximately 80% of human bacterial infections [3].

According to the literature, biofilm consist of a gelatinous polymer matrix adhered to a solid surface, consisted of microorganisms, extracellular polymeric substances (EPS), extracellular DNA (eDNA) and water. The extracellular polymers involve each bacterial cell forming a capsule that extends and brings all cells together in a cluster, which may increase the antimicrobial resistance in 1000 fold Balcázar, et al. [38]. Garnett and Matthews [39]. Interestingly, a bacterial biofilm may present one or several bacterial species, which is only a small part of this matrix [40]. These bacteria survive under the biofilm sub-optimal ambient conditions exhibiting antibiotic resistance, leading to persistent infections [41].

The formation of a biofilm is favored by numerous factors, including increasing nutrient concentration, and it protects bacteria against aggressive factors such as pH modification and host defenses [42]. The polymer matrix composition determines the physical-chemical and biological characteristics of this structure and the EPS composition varies depending on the environmental conditions and the bacterial strain [43]. In *S. aureus*, involvement of bacterial proteins are described, including surface proteins, such as autolysin (Atl), clumping factor

(ClfA and ClfB), binding protein and protein A, which are important in cell-cell interactions [44]. Differently, *P. aeruginosa* has a characteristic biofilm formed by several exopolysaccharides that play an important role in biofilm formation [45]. Current reports showed that treatment with DNases in the early stages of *P. aeruginosa* biofilm formation interferes with its maturation. This points to the importance of the extracellular DNA that is the major component of this structure and mediates cell-cell interactions [46].

The differences in density throughout the bacterial biofilm determine the gradients of nutrients and oxygen availability, resulting in differences in bacterial metabolic activity [43]. The proximity of the microorganisms inside the biofilm facilitates the dispersion of the resistance factors by horizontal gene transfer. This transfer occurs quickly due to an abundant bacterial subpopulation in biofilms named "persister", which are sleeper cells, present in the deeper areas of the biofilm [47]. These cells are highly tolerant to antimicrobial drugs, surviving despite the antibiotic use and adapted to a slow growth rate. The cell dormancy persister mechanism is not fully understood, but apparently is due to the expression of the toxin-antitoxin system (TA). When the antitoxin is degraded an imbalance occurs in this system, leading to activation of the toxin inducing a bacteriostatic effect [48]. It is clear that the exchange of genetic material among bacterial strains contributes to the emergence of resistance and plays a key role in the persistence of biofilms-associated infections [49].

There are some disadvantages to the bacteria for producing biofilms, but they are minors compared to the advantages. These disadvantages include difficult on transporting substances and metabolites in and out of the cell and the deviation of molecules for the production of extracellular polymeric substances over the cell metabolism pathways. This leads to the reduction of the bacterial growth rate specially when compared to the bacteria free planktonic form [50].

5. Bacterial Biofilm Production

Bacterial biofilms production is divided into the following steps: *i*) adhesion to the target surface, *ii*) secretion of intercellular adhesion molecules, *iii*) polysaccharide matrix secretion, *iv*) consolidation of the biofilms structure and *v*) secretion of molecules that allow dispersion and persistence of infection [51] (Figure 2).

The bacterial adhesion to the surface is a prerequisite to the biofilm formation process. This connection allows cell proliferation and biofilm formation [52]. The attachment of bacteria on the tissue or medical devices, in the human body is dependent on the interaction of bacteria with human proteins such as fibronectin and fibrinogen [53]. Teichoic acids are the major components of the cell surface of Gram-positive bacteria that contribute to biofilm formation. They are negatively charged and act as a primary or secondary factor in biofilm formation [54].

According to the literature, the accumulation phase seems to be dependent on adhesins and exopolysaccharides that promote adhesive interaction among bacterial cells [55].The staphylococcal polysaccharide intercellular adhesin called exopolysaccharide (PIA) or poly N –acetilglucosamine (PNAG) encoded by single operon, is an important component of the staphylococcal biofilm extracellular matrix [56].The partial deacetylation of this molecule produces positively charged residues that interact with the matrix component [57]. In addition, the group of surface proteins called MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) is also major determinants of the initial bacterial attachment on tissues and biomaterials.

P. aeruginosa produces three different exopolysaccharides (alginate, Psl and Pel) that participates in biofilm formation [58]. Alginates are composed of uronic acid and reduce the biofilm susceptibility to antibiotic and human defense [59]. The polysaccharide PSL is rich in mannose and galactose, and is involved in the initial connection and mature biofilm formation. It is produced during growth, mediating attachment to the surfaces and contributing to the formation of the microcolony [60]. Pel, which is similar to cellulose and rich in glucose, has been associated with rough colony phenotype. This exopolysaccharide plays a role in cell-cell interactions in *P. aeruginosa*, providing a structural support in the early stages of biofilm formation [61].

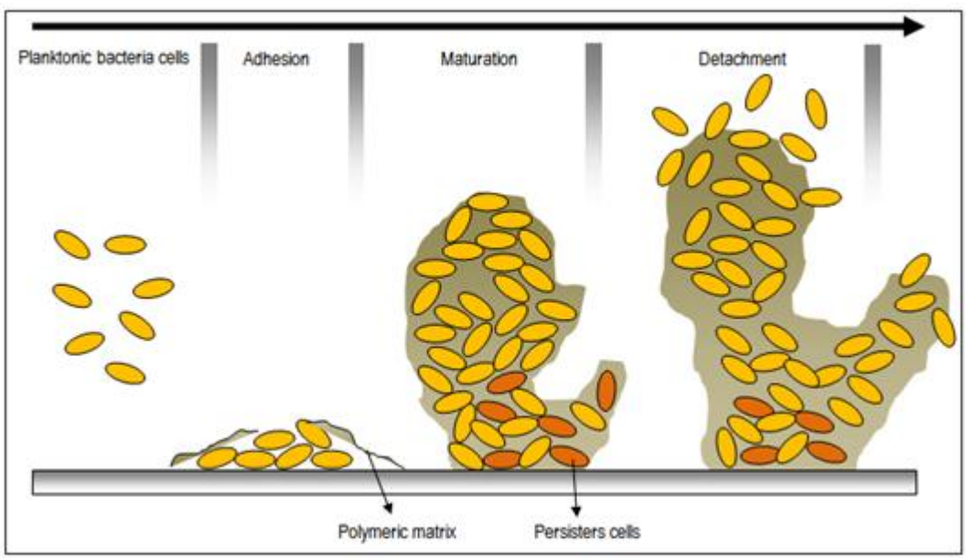

Figure-2. Biofilm production process.

Source: From the authors.

An important feature of biolfim is its fragility in the first/initial step formation as the initial adhesion is a reversible process. Unfortunately, the extracellular polysaccharide production and bacterial growth result in an

irreversible structure, where proteins, polysaccharides and nucleic acids act as a "glue" that brings the cells together [62]. In the *in vitro* growth, mature biofilms produce channels to provide nutrients to the biofilm deeper layer cells [63, 64] and once established, the biofilm becomes persistent and allows dissemination and bacterial infection [6].

Biofilm can be disassembled in individual cells or larger aggregates, through different and mechanical processes (Figure 2). Disassembly is crucial for spread bacteria to other colonization sites, and is triggered by blood flow, disruption of exopolysaccharides production, release of enzymes (proteases, glycosidases and DNases) and surfactants that degrade or solubilize the adhesion molecules in the biofilm matrix [65].

The surfactants act disrupting non-covalent interactions between cells and biofilm matrix molecules [66, 67]. The surfactant molecules responsible for staphylococci biofilm maturation are soluble phenol (PSMs) that are amphipathic molecules [68]. In *P. aeruginosa*, the surfactants are controlled by "quorum sensing" (QS), a cell-cell communication mechanism that controls the bacterial gene expression and phenotypic characteristics [69]. This bacterium is mobile and the dispersion may begin with the up-regulation of motility [70].

Biofilm maturation occurs through an enzymatic degradation of the biofilm matrix components, mostly proteases and nucleases, especially in staphylococci. Some of these enzymes are under the control of QS [71, 72]. The cell dispersion process takes place through the QS that allows the formation of small agglomerates and a new biofilm formation [73].

6. Regulation of Biofilm Formation: A Molecular View

The literature reports that the activation of biofilm formation depends on the site of infection. The regulation of this mechanism involves c-di-GMP (cyclic guanosine monophosphate dimer). This second messenger is a major participant in the biofilm formation that requests more than 100 proteins. This signaling network is especially prominent in proteobacteria such as *P. aeruginosa* [74].

The most important role of c-di-GMP is to regulate the activation of biofilm formation and motility. According to the literature, the c-diAMP control system of Gram-positive bacteria such as staphylococci has an essential role in resistance to antibiotics, bacterial stress, virulence and biofilm formation [75].The activation of this system includes the regulation of several physiological processes through binding of c-di-GMP transcription regulators and the activation of biofilm related genes. The expression of the extracellular matrix components such as adhesins, exopolysaccharides, extracellular secretion system, DNA, with cell death and decreased motility, result in a three dimensional structure of the mature biofilm. In *P. aeruginosa*, this messenger is related to the production of alginate that contributes to the biofilm persistence [76].

Biofilm activation may also occur via signaling (p) ppGpp, which acts in synergy with c-di-GMP. This process is considered a regulatory response to the global stress of the environment adversity. This reduces the basic cellular functions such as protein synthesis, cell division and cell wall synthesis and induces protective responses against oxidative and osmotic stress [77].

The literature has shown that the (p) ppGpp signaling pathway coordinates the expression of virulence factors required at different stages during acute and persistent infections. It contributes to the initial adhesion to host tissues and intracellular survival and is involved in controlling the formation and maturation of the biofilm. In most studies, a clear reduction in biofilm formation is detected in the absence of (p) ppGpp, confirming its role in protecting against environmental stress [78].

7. Biofilm and Quorum Sensing

Quorum sensing is a signaling system that detects microbial population density and different external conditions, and control the biofilm bacterial population and gene expression. This mechanism is essential to the formation of bacterial biofilms [79]. A Gram-negative mutant bacteria with a defective QS is unable to form biofilm in comparison to the wild type.

Different species use different molecules, but some species share related QS systems, such as *P. aeruginosa* and *S. aureus*. This communication is made up of small molecules secreted by bacterial cells called autoinducing peptide (AIP) [80, 81]. In staphylococci, these autoinducers are modulated by a complex gene *agr* (accessory gene regulator) and also by complex *sarA* and *rnaIII* [82]. The *agr* complex modulates several virulence factors related to the adhesion and growth processes and includes adhesins, fibronectins among others [83].

Together, this *agr* locus has a two-component regulatory system that will secrete one autoinducer molecule. This signaling with regulatory elements (e.g. RNAIII), lead to changes in the gene expression, increasing virulence factors and decreasing MSCRAMMS, including protein A and fibronectin binding protein [84].

In *P. aeruginosa*, the QS communication system is more complex and consists of two systems: LAS and AHL dependent RHL (acyl-homoserine lactone). This system produces LAS signaling molecule 3-oxo-C12-HSL (N-3-oxo-dodecanoyl-homoserine lactone). The expression of virulence factors, including elastase, protease and exotoxin A, is modulated by the LAS system that also acts as a determinant of virulence. It modulates the host defense responses by inhibiting the activation of dendritic cells and T cells. This system also promotes apoptosis of neutrophils and macrophages eliciting the production of inflammatory cytokines in a calcium dependent manner [85, 86]. The RHL system acts through C4-HSL (N-butanoylhomoserine lactone), responsible for the production ramnolipids and elastase. The LAS and RHL systems are still modulated by a quinolone, 2-heptyl-3-hydroxy-4-quinolone, called PQS, which increases the complexity of QS network [87, 88].

8. The Effects of Antibiotics and Immune System on Bacterial Biofilms

Despite the biofilm polymeric matrix acts as a barrier that slows the diffusion of antimicrobial agents, protecting it from antibiotics and host immune system [89] studies have shown that the introduction of antibiotics into the bacterial biofilm modifies the bacterial metabolism, their cell-cell communication, its virulence and the biofilm

formation [90, 91]. Polymorphonuclear (PMN) are the first cells to reach the site of infection, attracted by the cytokines. Interestingly, some bacteria have chemoattractants, such as the tripeptide f-Met-Leu-Phehomoserine lactone, a quorum sensing molecule from *P. aeruginosa* [81, 92].

In the infected site, PMN recognizes molecules associated with pathogens (PAMPs) through Toll-like receptors and interacts with lipopolysaccharides, complex carbohydrates, bacterial DNA, among others [93]. PMN acts through phagocytosis but with little or no activity due to the alginate. This exopolysaccharide is related to structural changes in the biofilm, increasing its resistance. Taken together, these data point to the EPS role as an important biofilms "defense mechanism" against the immune system [94].

Rhamnolipids are amphiphilic molecules composed of fatty acid such as the thermo stable hemolysin of *P. aeruginosa*. The synthesis is controlled by the quorum sensing mechanism and the role of these molecules is to maintain the biofilm structure, creating channels for nutrients, and further extracellular components to avoid leukocytes attack [95]. According to the literature, there are different interactions depending on the bacterial biofilm. For example, neutrophils remained immobilized in the biofilm of *P. aeruginosa* but not in *S. aureus*, where they do not display a phagocytic activity [43].

After the biofilm is formed, the human defense mechanisms are difficult to act. The oxidative species, or the reactive species of chlorine produced by the host immune response (e.g. hypochlorite or chlorine dioxide) cannot diffuse through the matrix [96]. However, lactoferrin is stored in neutrophils, and is present in external secretions. It is capable of preventing biofilm formation due to the chelating ability of lactoferrin ions. Another molecule also expressed by neutrophils is catelecidine, which is expressed primarily by epithelial cells, but also found in body fluids. This molecule also inhibits the biofilm formation by reducing bacteria adhesion, stimulating motility, and down regulating quorum sensing genes required for biofilm formation [97].

An important aspect of the biofilm formation is the initial attachment of bacteria to the surface. *S. aureus* adhere more in the metals used for implants and prostheses in comparison with the conventional plastic culture dishes. Therefore *S. aureus* biofilm formation occurs more rapidly on metals, especially on rough surfaces [81, 98].

9. Biofilm and Bacterial Advantages

According to the literature, one of the main advantages of bacteria producing biofilm is the resistance against antimicrobial agents, which difficult to eradicate infections. The biofilm shows maximum resistance to antibiotics in the mature stage due to several mechanisms [99, 100] including:

(1) Low diffusion of antibiotics over the polysaccharide biofilm matrix
(2) Bacterial changes, including the slow growth rate and differences in the concentrations of oxygen, nutrients or environmental stress;
(3) Phenotypic changes;
(4) Quorum-sensing;
(5) Efflux pumps expression;
(6) Presence of persister cells that resist killing when exposed to antimicrobial agents.

Biofilms present higher proteolytic activity than bacterial planktonic counterpart and the biofilms-producing bacteria become more virulent and with a greater ability of causing tissue destruction [101]. Clinical studies showed increased disease severity when using subtherapeutic doses of antibiotics or inadequate use (eg. Lower period of time) on biofilms-producing bacteria [102].

The efflux pump resistance is a key mechanism of biofilms-producing Gram-negative bacteria as it regulates the bacterial internal environment, removing toxic substances, including antimicrobial agents and metabolites. The literature reports that some efflux pumps have higher expression in biofilm producing bacteria than in the planktonic bacterial form [103].

As biofilms are composed of subpopulations of metabolically active and inactive cells, these phenotypes subsets show different susceptibility to antibiotics. The active subpopulations demonstrate antibiotic sensitivity, while inactive subpopulations are resistant to these compounds. More studies are needed to elucidate the mechanisms by which these antibiotics induce changes in these biofilms-producing bacteria strains [47].

The inherent resistance of biofilms to antibiotics and host immune responses makes the eradication on medical devices more difficult. Currently biofilm infections are treated by removal or replacement of the infected medical devices, combined with antibiotic therapy [104].

According to some authors the first 6 hours after implantation of invasive devices is the most critical period not only to prevent biofilm formation but also to invest in the long-term success of an implant. Data suggest that inhibition of biofilm formation within a short period in a the infection early phase could be beneficial [49].

Since some bacteria can produce a different biofilm phenotype in response to different surfaces, also reducing sensitivity to antibiotics [105] one alternative may be coating the device surface with a bactericide reagent such as antibiotics. This approach is effective, but increases the risk of antibiotic resistance. In addition, sub-inhibitory concentrations of antibacterial agents may enhance the formation of biofilms, which may increase the resistance problems [106].

Another approach is to coat the surface with inert reagents to prevent the adhesion of bacteria such as plasma coating technology (TMS). TMS inhibits the bacterial binding, preventing maturation of the biofilm [107]. Apparently, TMS increase bacterial susceptibility to certain antibiotics such as ciprofloxacin, reducing the bacteria count over 99%. However to be effective it is necessary that the bacterial load is reduced by three units, which limits their potential for clinical application [49].

Based on this context, new antibacterial agents are urgently needed for the removal of biofilms-producing bacteria that are currently highly resistant to the traditional antimicrobial agents [17]. Although the mechanisms of antibiotic resistance in the biofilm are not yet fully understood, the inability to successfully treat infections with conventional therapies increases the demand for research and development of new treatment strategies [108]. After

the "golden age" when the largest number of antibiotics was discovered, there was a decrease in production of these drugs. Since 2000, twenty-two new antibiotics were launched worldwide [109]. Among these, only five new classes of antibiotics: Linezolid (systemic, approved in 2000), Daptomycin (systemic, approved in 2003), retapamulin (topic, approved in 2007), fidaxomicin (specific, approved in 2010) and Bedaquiline (systemic approved in 2012) - the latter two are the first representatives of their classes [110].

New treatments are needed to combat bacterial biofilm associated diseases. Conventional antibiotics have limited effectiveness against biofilms-related infections and contribute to the increase of multidrug resistant cases [111]. Therefore an organized and network effort should be developed to identify new therapeutic agents with anti-biofilm activity. Lately, biophosphocins [112] antimicrobial peptides [113] new antibacterial coating strategies [114] and nanotechnology associated to antimicrobial therapy [115] have been described, but should be further tested to avoid and/or reduce the incidence of infections associated with biofilm formation, hoping to improve billions of human lifes.

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