Mechanisms of Biofilm Development, Antibiotic Resistance and Tolerance and Their Role in Persistent Infections

Divya Srivastava, Suchi Srivastava, Poonam C. Singh, and Adesh Kumar

Abstract

Bacteria frequently form biofilms in response to stress factors that include exposure of planktonic cells to subinhibitory concentrations of antibiotics. When these attach to a surface, they switch to the biofilm mode of growth and undergo a phenotypic shift in behaviour. During this process, a large suite of genes are differentially regulated to develop a biofilm, which protect them from killing by antibiotics. This leads to the persistence of biofilm infections and the mechanisms used to protect bacteria in biofilms distinct from those that are responsible for conventional antibiotic resistance as well as tolerance. This tolerance to antibiotics is contributed to by multiple factors such as poor antibiotic penetration, nutrient limitation adaptive stress responses, slowed metabolism and the formation of persister cells. The present chapter deals with the introduction to biofilm and their mechanism to achieve antibiotic resistance as well as tolerance properties including their role in persistent infection with some advancement in biofilm research.

Keywords

Biofilm · Tolerance · Persistence · Antibiotic resistance · Challenge in chemotherapy

D. Srivastava · A. Kumar (✉)
Narendra Deva University of Agriculture and Technology, Faizabad, Uttar Pradesh, India

S. Srivastava · P. C. Singh
CSIR- National Botanical Research Institute, Lucknow, Uttar Pradesh, India

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1 Introduction to Biofilm

A biofilm is community of microorganisms where cells are stuck to each other and often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of extracellular polysaccharides also called extracellular polymeric substances (EPS) (Chakraborty et al. 2018). Cells associated with a biofilm produce a polymeric conglomeration of EPS, DNA and proteins. They have been metaphorically described as "cities for microbes" as they have three-dimensional structure and represent a community lifestyle for microorganisms.

Biofilms may get developed on any surface including living as well as non-living surfaces. They can be prevalent in natural, industrial, hospital settings and public sectors. The new microbial cells in a biofilm are not as physiologically same as planktonic cells of the same organism. Unlike biofilms, planktonic cells are single cells that may float or swim in a liquid medium (O'Toole and Kolter 1998).

Microbes are governed by many different factors to form biofilms (O'Toole and Kolter 1998) which may include cellular recognition of specific as well as nonspecific attachment sites on a surface or by the exposure of planktonic cells to subinhibitory concentrations of antibiotics (Hoffman et al. 2005). Cells undergo a phenotypic shift in behaviour in which large suites of genes are differentially regulated when they switch to the biofilm mode of growth (An and Parsek 2007).

A biofilm may be considered a hydrogel that is a complex polymer containing many times its dry weight in water. Biofilms are entire biological systems where bacteria organize themselves into a coordinated functional community. These biofilms can attach to a surface such as a tooth, rock or virtually any surface and may include a single species or a diverse group of microorganisms. A biofilm usually begins to form when a free-swimming bacterium attaches to a surface, multiples and begins to produce EPS.

1.1 IUPAC Definition of Biofilm

"*Aggregate* of microorganisms in which all cells that are embedded within a self-produced matrix of extracellular polymeric substances (EPSs) adhered to each other and/or to a surface." EPS is a polymeric conglomeration which is composed of extracellular *biopolymers* in various structural forms and also referred to as slime (Vert et al. 2012).

1.2 Formation of Biofilms

The formation of a biofilm begins when free-floating microorganisms attach to a surface and/or adhere to each other (O'Toole and Kolter 1998). This process starts with the first colonist bacteria, which adheres to the surface initially through weak, reversible adhesion via van der Waals forces (Danhorn and Fuqua 2007). If the

colonists are not separated from the surface, then they can anchor themselves permanently using pili which are cell adhesion structures.

Bacteria with high hydrophobicity may have reduced repulsion between the bacterium and the extracellular matrix (Danhorn and Fuqua 2007), *reducing biofilm formation. While a* few species of bacteria are not able to attach to a surface of their own due to their limited motility, they may be able to anchor themselves onto the matrix or to bacteria colonists. In general, non-motile bacteria cannot recognize surfaces or aggregate together as easily as motile bacteria (Danhorn and Fuqua 2007).

During surface colonization, bacterial cells are capable of communicating using quorum-sensing (QS) signals such as N-acyl homoserine lactone (AHL). Quorum sensing (QS) enables transfer of information connected with biocidal agents' resistance and the mechanisms of their activation. Thus, biofilm-forming bacteria are the cause of many chronic, recurrent and persistent infections (Karthik et al. 2018). Once colonization has taken place, a combination of cell division and recruitment leads to further biofilm maturation. Matrices cover the entire bacterial biofilms with polysaccharides and some other component which may also contain material including components of blood as erythrocytes and fibrin and soil particles. The final stage of biofilm formation is also known as dispersion where the biofilm is completely established and planktonic cells may be released either actively or passively.

Steps Involved

Following are the five major stages of biofilm development (Monroe 2007):

1. Attachment
2. Microcolony formation
3. Three-dimensional structure formation and maturation
4. Detachment

1.2.1 Attachment

Bacteria make a reversible connection with the surface and/or already adhered other microbe to the surface to form biofilms and a solid–liquid interface develops at that place. This interface can provide an ideal environment for microorganism to attach and grow (e.g. blood, water) (Costerone et al. 1999). Rough, hydrophilic and coated surfaces will provide better environment for biofilm formation. Nutrient concentrations, flow velocity and temperature play an important role in increased attachment; apart from these factors, occurrence of locomotor structures on cell surfaces is also important and may possibly provide an advantage in biofilm formation when there are mixed community and these structures include flagella, pili, fimbriae, proteins or polysaccharides (Donlan and Costerton 2002).

1.2.2 Microcolony Formation

As bacteria get adhered to the physical surface or some biological tissue with a stable binding, formation of microcolony takes place. This microcolony is developed from multiplication of bacteria in the biofilm which starts as a result of

chemical signals. The production of exopolysaccharide is activated at molecular level production of exopolysaccharide is activated when intensity of the signal cross certain threshold; therefore the bacterial cell divisions take place within the embedded exopolysaccharide matrix, which finally result in microcolony formation by this way using such chemical signal (Mckenney 1998).

1.2.3 Three-Dimensional Structure Formation and Maturation

The stage of microcolony formation to develop biofilms is followed by expression of certain biofilm-related genes, and these gene products are required for the formation of EPS as the main structural material of biofilm. It has been reported that bacterial attachment by itself can trigger formation of extracellular matrix which is later followed by water-filled channels formation for transport of nutrients within the biofilm. Researchers have proposed that these water channels are like circulatory systems, distributing different nutrients to microcolonies and also removing waste materials from the communities in the microcolonies of the biofilm (Parsek and Singh 2003).

1.2.4 Detachment

Once the biofilm formation is completely done, the researchers have often noticed that bacteria present in microcolony now leave the biofilms itself on regular basis, and by doing this, the bacteria can undergo rapid multiplication followed by dispersal. A natural pattern of programmed detachment occurs for the detachment of planktonic bacterial cells from the biofilm. Due to some mechanical stress, it has been reported that sometimes the bacteria get detached from the colony into the surrounding while in most cases some bacteria stop EPS production and they get detached into environment. There are two ways for dispersing of biofilm cells; it may occur by either detachment of new formed cells from growing cells or dispersion of biofilm aggregates due to flowing effects or due to quorum sensing (Baselga et al. 1994). In biofilm, cells are removed from surface due to an enzyme action that causes alginate digestion. Phenotypic characters of organisms are apparently affected by the mode of biofilm dispersion. The dispersed cells have the ability to retain certain properties of biofilm like antibiotic insensitivity. The cells after getting dispersed form biofilm due to growth return quickly to their normal planktonic phenotype.

2 Properties of Biofilms

Biofilms are present as solid substrates submerged in or exposed to an associated solution. Once microorganisms get enough nutrients, a biofilm can quickly grow to be megascopic (visible to the naked eye). These biofilms consist of living organisms like bacteria, archaea, protozoa, fungi and algae; each group performs specialized metabolic functions. The social organization (cooperation/competition) within a biofilm depends on the various species present (Nadell et al. 2009).

3 Extracellular Matrix

The extracellular matrix consists of exopolysaccharides (EPS), proteins and nucleic acids (Branda et al. 2006). A large proportion of this matrix consisting EPS is more or less strongly hydrated; however, cellulose is one example of hydrophobic EPS which is produced by a range of microorganisms. The cells are encased by the matrix and present within the matrix and also facilitate communication among them through gene exchange as well as biochemical signals. Along with the cells, the EPS matrix also traps extracellular enzymes to keep them in close proximity to the cells; therefore, this matrix shows an external digestion system, and it allows for stable synergistic micro-consortia composed of different species (Wingender and Flemming 2010). Some biofilms contain water channels to facilitate nutrients distribution and signalling of biomolecules (Stoodley et al. 1994). This EPS containing extracellular matrix is strong enough; under certain conditions, biofilms can become fossilized (stromatolites).

Bacteria living in biofilms display different phenotypes from free-floating microorganisms of identical species, because the dense and guarded atmosphere of the biofilm permits them to associate and move in different ways (Vlamakis et al. 2008). One major advantage biofilms provide is an increased tolerance to detergents and antibiotics. This is due to many factors, including the dense extracellular matrix and outer layer of cells that shield the inside of the community. In some cases, antibiotic tolerance is increased a thousandfold (Stewart and Costerton 2001). Horizontal gene transfer is commonly expedited inside biofilms (Chimileski et al. 2014), which can increase the sharing of antibiotic resistance mechanisms (Molin and Tolker-Nielsen 2003). Extracellular DNA is also a major structural element of biofilms (Jakubovics et al. 2013) *and is therefore subject to* enzymatic degradation, which can weaken the biofilm structure and unharness microbial cells.

However, it has been observed that biofilms are not always less susceptible to antibiotics like the biofilm form of *P. aeruginosa* has no greater resistance to antimicrobials than do stationary-phase planktonic cells, although when the biofilm is compared to logarithmic-phase planktonic cells, the biofilm does have greater resistance to antimicrobials. This resistance to antibiotics in both stationary-phase cells and biofilms may be due to the presence of persister cells (Spoering and Lewis 2001).

4 Diversity of Biofilm Based on Taxonomy

Many different types of bacteria form biofilms. Some examples of biofilm-forming gram-negative species are *Pseudomonas aeruginosa or Escherichia coli*, while biofilm-forming gram-positive bacteria are *Listeria monocytogenes*, *Staphylococcus* spp., *Bacillus* spp and lactic acid bacteria, including *Lactobacillus plantarum* and *Lactococcus lactis*. Some bacteria form biofilms in aquatic environments like *Cyanobacteria* (Danhorn and Fuqua 2007).

Biofilms are formed by bacteria that colonize plants, e.g. *P. putida*, *P. seudomonas* and other related pseudomonas which are common plant-associated bacteria found on leaves, roots and in the soil, and the majority of their natural isolates form biofilms. Several nitrogen-fixing symbionts of legumes such as *Rhizobium leguminosarum* and *Sinorhizobium meliloti* form biofilms on legume roots and other inert surfaces (Joubert et al. 2006).

Along with bacteria, biofilms are also generated by archaea and by a range of eukaryotic organisms, including fungi, e.g. *Cryptococcus laurentii* (Joubert et al. 2006), and microalgae. Among microalgae, one of the main progenitors of biofilms are diatoms, which colonize both fresh and marine environments worldwide (Carl et al. 2014; Aslam et al. 2012).

5 Mechanism of Antibiotic Resistance

Bacterial biofilms are known for their tolerance to antibiotics and their reactive molecules which are produced by the host immune systems than planktonic bacteria. It has been estimated that the antibiotics resistance in biofilm cells can be up to 10,000 times more than the antibiotics resistance in planktonic cells (Costerton et al. 1995; Nickel et al. 1985). The antibiotic resistance was observed when Costerton and co-workers treated *P. aeruginosa* biofilm and planktonic cells with tobramycin, and then they found the planktonic cells could not survive greater than 50 μg/ml tobramycin whereas the biofilm cells could tolerate 1 mg/ml tobramycin (Nickel et al. 1985). Later, Abee and co-workers reported that with two different disinfectants, benzalkonium chloride and the oxidizing agent sodium hypochlorite, the effective inhibitory concentrations on *S. aureus* biofilms are 50 and 600 times higher than planktonic cells, respectively (Luppens et al. 2002). The reason(s) for increased antibiotic resistance by bacterial biofilms is/are not yet fully understood, but it is highly probable that multiple factors work together to protect biofilm cells from antibiotic treatment. Some of the possible mechanisms for antibiotic resistance exhibited by bacterial biofilms are discussed below.

5.1 EPS Matrix Protection

EPS matrix plays an important role in antibiotic resistance by limiting antimicrobial agents' penetration into the biofilm. Charged polysaccharides and eDNA can trap several kinds of antibiotics. The penetration property of antibiotics has been measured by the concentration at the base of the biofilm by Suci and co-workers. Results showed that ciprofloxacin concentration in *P. aeruginosa* biofilm was dramatically reduced, but not completely blocked (Suci et al. 1994). Steward and co-workers investigated the penetration limitation of ampicillin and ciprofloxacin on *K. pneumoniae*. Ciprofloxacin has a

much better penetration capability than ampicillin. As a result, biofilm cells could tolerate concentrated ampicillin, but their resistance to ciprofloxacin is poor (Anderl et al. 2000).

5.2 Horizontal Gene Transfer

Some bacteria can acquire antibiotic resistance via random mutations on genes. Others also harbour antibiotic-resistant genes on plasmids. Plasmids can be easily passed on to other cells by horizontal gene transfer. In biofilms, the frequencies of horizontal plasmid transfer are much higher than between planktonic cells. Studies on *S. aureus* biofilms showed that biofilms promote the spread of plasmid-borne antibiotic resistance genes by conjugation/mobilization (Savage et al. 2013).

5.3 Reduced Growth Rate

There is limited availability of oxygen and nutrients inside biofilms, so biofilm cells, especially those in the deep layers, have a slow metabolic rate, as well as growth rate and division rate. These features make biofilm bacteria insensitive to antibiotic drugs that target dividing cells. For example, the targets of β-lactams are dividing cells, so when they are used on *E. coli* biofilms, their bacteriolytic activity is diminished (Ashby et al. 1994).

5.4 Persister Cells

In biofilms, there is a small subpopulation of cells called persister cells (Lewis 2007; Keren et al. 2004). Their growth rate is zero or extremely slow. Most of the antibiotics that are currently used in the clinic, which target processes that are relevant for cell growth or division, are not effective against persister cells. Therefore, these cells act as disease reservoirs that could reactivate into infectious particles once the antibiotic stress has been removed.

5.5 Efflux Pumps

Efflux pumps allow bacteria cells to pump intracellular toxins out, including antibiotic drugs. Efflux pumps are also expressed in planktonic cells, but some efflux pump genes are upregulated in biofilm, indicating that they contribute to antibiotic resistance. Zhang and co-workers identified a novel *P. aeruginosa* efflux pump gene PA1874–1877, and the expression level of PA1874–1877 gene in biofilm is much higher than in planktonic cells (Zhang and Mah 2008). Efflux pump encoded by this gene increases the resistance to tobramycin, gentamicin and ciprofloxacin.

5.6 EPS Matrix Protection

EPS matrix provides physical protection to the aggregated biofilm cells. Lei and co-workers showed that exopolysaccharide alginate in *P. aeruginosa* biofilms kept biofilm bacteria from human leukocyte killing (Leid et al. 2005).

6 Mechanism of Antibiotic Tolerance

The antibiotic tolerance mechanisms in biofilms include failure of antibiotics to penetrate biofilms (Jolivet-Gougeon and Bonnaure-Mallet 2014), slow growth rate, altered metabolism, persister cells, oxygen gradients and extracellular biofilm matrix (Römling and Balsalobre 2012).

6.1 Failure of Antibiotics to Penetrate Biofilm

Antimicrobials may be prevented from penetrating the biofilm by its matrix acting as a barrier. Although prevention of penetration is no longer believed to be a significant factor, antibiotics can be prevented from penetrating if they bind to components of the biofilm matrix or to bacterial membranes (Walters et al. 2003; Chiang et al. 2013). Positively charged antibiotics such as aminoglycosides and polypeptides that bind to negatively charged biofilm matrix polymers are delayed in their penetration through biofilm. Besides, it is possible for biofilm to find retention places on medical or dental devices, where it is protected from antibiotics. It should also be recognized that the high density of bacteria in biofilms increases the selection of resistant bacteria under pressure from antibiotics by enhancing horizontal gene transfer and the frequency of mutation (Lazăr and Chifiriuc 2010).

6.2 Nutrient-Deficient Environment

Multiple microcolonies in the biofilm create a metabolically heterogeneous bacterial population (Hall-Stoodley et al. 2004; Lenz et al. 2008). In such environments, local diffusion gradients are developed, causing anoxic and acidic zones in the interior of the biofilm (de Beer et al. 1997). Zones that are nutrient-deficient can produce stationary phase-like dormant cells, which may be responsible for the general resistance of the biofilm to antibiotics (Walters et al. 2003; Fux et al. 2004). It is thought that limited penetration of nutrients rather than restricted access for antibiotics contribute to the general tolerance seen in biofilms towards antibiotics (Hall-Stoodley and Stoodley 2009).

6.3 Altered Metabolism

Within the biofilm population, cells with diverse genotypes and phenotypes coexist. This implies that distinct metabolic pathways are expressed based on the local environmental conditions in the biofilm. The metabolic activity of bacteria is high in the outer part of the biofilm, while it is low in the inner part (Werner et al. 2004; Pamp et al. 2008). The difference in physiologic activity is caused by limited oxygen and nutrient penetration due to consumption by bacteria present (Hall-Stoodley and Stoodley 2009). When bacteria grow in a biofilm, they may experience antibiotic tolerance through nutrient deprivation, which causes slow bacterial growth or starvation (Xu et al. 2000). Many antibiotics are directed against processes occurring in growing bacteria, such as replication, transcription, translation and cell wall synthesis. Therefore, increased antimicrobial tolerance will occur in biofilm bacteria with low metabolic activity located in the inner part of biofilms (Ciofu et al. 2014). As an example, the starvation of most amino acids, especially leucine, cysteine and lysine, or of glucose was found to induce biofilm tolerance to ofloxacin (Hall-Stoodley and Stoodley 2009).

6.4 Oxygen Gradients

The oxygen tension in the depth of biofilm is low. In *P. aeruginosa*, hypoxia increased antibiotic resistance through altering the composition of multidrug efflux pumps (Schaible et al. 2012). Microenvironmental hypoxia contributes significant development of antibiotic resistance in *P. aeruginosa* infecting cystic fibrosis patients. It has been found that in *P. aeruginosa* biofilms grown in vitro for 48 h with different antibiotics, oxygen limitation accounted for 70 % (depending on the antibiotic) or more of all the antibiotic tolerance (Borriello et al. 2004). It was found that oxygen penetrated about 50 μm into biofilm with an average thickness of 210 μm. 48-h colony biofilms were physiologically heterogeneous and most of the cells occupied an oxygen-limited stationary phase. The anaerobic environment within biofilms will most likely affect aminoglycoside antibiotic activity due to the downregulation of energy metabolism genes (Kindrachuk et al. 2011) and by triggering changes in gene expression (Taylor et al. 2014).

6.5 Presence of Persister Cells

Till date, there are no antibiotics that can kill all the pathogenic microorganisms; some organisms are always left unaffected after antibiotic treatment so-called persisters. This is a small subpopulation of bacteria that has entered a slow-growing or starving state and that is highly tolerant to be killed by antibiotics (Lewis 2012;

Hu and Coates 2012). The reduced metabolic rates of these cells make them less sensitive to antibiotics compared to active, exponential growth-phase bacteria. Because antibiotics must work on growing cells to destroy them, the hibernating cells can outlast the antibiotic and then repopulate the infectious site. This occurs especially in sites where immune components are limited, such as in biofilm. Thus, persisters are considered to be significant contributors to the persistence of biofilm infections.

7 Role of Biofilm in Persistent Infections

Nowadays, persistent infections have become a global challenge for human beings, claiming millions of lives every year and demanding huge medical and social resources. The host suffering tries to eliminate a pathogen while the pathogen tries to survive in the host; therefore, the development of persistent infections has been exemplified as a game of "Cat & Mouse". The simplest survival strategy employed by bacteria pathogens is to form a biofilm which is an amorphous and dynamic structure, and this biofilm is not only resistant to antibiotics but also resistant to host immune clearance. The biofilms provide an important reservoir of cells that can repopulate colonized sites and can lead to bacterial infections caused by different bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*) in cystic fibrosis pneumonia (Singh et al. 2000), *Escherichia coli* (*E. coli*) in urinary tract infections (Anderson et al. 2004) and *Mycobacterium tuberculosis* (*M. tuberculosis*) in human tuberculosis (Ojha et al. 2008). Biofilms are also responsible for persistent *Streptococcus mutans* (*S. mutans*) infections on tooth surfaces and the most nosocomial infections are persistent biofilm infections (Costerone et al. 1999; Costerton et al. 2003). It is estimated that, in developed countries, over 60% of treated infectious conditions are caused by biofilm formation. As a correlation between biofilm formation and bacterial persistence has been established (Balaban et al. 2004), the possibility of using drugs targeting biofilm formation in combination with the current antibiotics is emerging as a potential therapeutic approach for this type of bacterial persistent infection. Several anti-biofilm and/or biofilm control strategies, such as anti-adhesion, quorum-sensing disruption and selective targeted antimicrobial peptides, have been recently developed.

8 Advancements in Biofilm Research

Mostly it has been observed that the biofilm formation of infectious significance is found on "implant devices". The 2nd most common reason for ventilator-associated pneumonia (VAP) and catheter-associated urinary tract infections (CAUTI) was found due to *P. aeruginosa* which forms biofilms on endocardial tubes and catheters in CAUTI and VAP patients (Muhsin et al. 2018). Whereas other recent advancement in biofilm research has been applied to control energy crises as this approach is using microbial fuel cells (MFCs). These MFCs produce electricity, and they are

utilizing chemical energy found in organic and some inorganic compounds. Electrogenic microbes play a role in this process by accepting or donating electrons to an external object (electrode), while some non-electrogenic microbes are also involved as part of a synergistic electrogenic biofilm.

Another biofilm-related problem is caused by *Asaia* species, which form biofilms on plants used for the production of soft drinks, and may thereby contaminate the soft drinks even in the presence of a preservative. Additionally, biofilm resistance against antibiotics has reached an alarming state. Antibiotic therapy is not effective once the biofilm is matured. The Chinese medical herb i.e. *Herba patreniae* which degrades the mature biofilm of *P. aeruginosa* and its exopolysaccharide. A biofilm-growing mucoid strain can play a role in the exacerbation of cystic fibrosis. There are several antimicrobial agents used to treat this biofilm-forming strain of *P. aeruginosa*. For example, Ciprofloxacin has been shown to kill the bacteria found on the surface of the biofilm, whereas Colistin was shown to kill the ones found in the depth of the biofilm. There may also be many other possibilities that can be applied in the journey of treatment of biofilm-related infections some examples can be like inhibiting quorum sensing through breaking of matrix by alginate *lyase* or F-actin. For bacterial biofilm formation, quorum-sensing activity is very important, as revealed by genetic analysis of biofilms. There are many identified molecules produced by eukaryotes and prokaryotes to quench the quorum sensing, i.e. quorum quenching (Muhsin et al. 2018; Paramasivam et al. 2017).

9 Conclusions

Biofim formation is a two-stage biological process which is controlled by surface adhesions and cell-to-cell communication pathways. The aggregated bacterial cells which are protected and/or coated by extracellular matrix are insensitive to both nutritional stimulation as well as hostile attacks. In the human body, biofilms may trigger persistent infections with chronic inflammation. There is no single mechanism for antibiotic-induced biofilm tolerance or resistance, although a global response to various forms of stress may be a significant denominator. Probably, multiple mechanisms of tolerance and resistance act together, causing an increased overall level of resistance and tolerance. In this context, newly recognized genes for biofilm-detected antibiotic tolerance and resistance seem to be particularly important fellow players. Most studies on biofilm-induced resistance have been undertaken with *P. aeruginosa*. Such studies, although fundamental for our dawning knowledge of biofilm-specific antibiotic tolerance and resistance as such, may have limitations. The results from *P. aeruginosa* cannot uncritically be extrapolated to other forms of biofilm-induced chronic diseases which, often, are affected not by biofilms of single but of multiple species, where cells are subjected to a plethora of signals. However, *P. aeruginosa* can serve as a valuable model system for delineating biofilm-specific antibiotic tolerance and resistance mechanisms, and the results achieved with this bacterium should initiate studies on multispecies biofilm models in vitro and in vivo. A major setback in the treatment of

biofilm-related infections is the ineffectiveness of existing antibiotics due to the protective layers built by cells in the biofilm. There is therefore limited antibiotic penetration, so the community of sedentary cells persists even in the presence of antibiotics effective against their motile counterparts. The dispersion of matured biofilms may primarily require the disruption of the EPS matrix. Components of the EPS matrix such as alginate, Pel and Psl in *P. aeruginosa* biofilms could, therefore, be vital targets in the disruption of biofilm structure. With cells exposed, the drug compound may then exhibit bactericidal activity or act by dispersing the cells. On the other hand, the formation of new biofilms could be inhibited by preventing initial processes like attachment of cells to surfaces. This would be crucial in the development of medical and dental implants as they are easily colonized by biofilm-forming pathogenic bacteria. Nevertheless, the gap between observations made from an in vitro model and an in vivo model continues to be problematic. Choice of animal model, depending on how closely related the model is to humans, would be a critical parameter in the successful development of any anti-biofilm drug. Biofilm formation on indwelling medical devices greatly affects surgical and instrumental procedures and public health as well. It also has implications in non-device-related human-health complications. There is a need for an in-depth research to optimize measures for its prevention. Good hygienic conditions and practices are very necessary to avoid biofilm formation. With the passage of time, and with the advent of new technologies, progress has been made to remove and control biofilm-associated infections. However, new anti-biofilm strategies are necessary to handle biofilm-associated chronic infections.

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