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Antimicrobial resistance and biofilm formation of *Pseudomonas aeruginosa*: a short review article

Abdelraouf A. Elmanama[1], Suhaila A. Al-Sheboul[2], Renad I Abu-Dan[1]

1 Islamic University of Gaza, Faculty of Health Sciences Medical Laboratory Sciences Department, Gaza, Palestine.
2 Jordan University of Science and Technology, Faculty of Applied Medical Sciences, Department of Medical Laboratory Sciences, Irbid, Jordan.

Contact information:

Prof. Abdelraouf A. Elmanama.

Address: Medical laboratory Sciences Department, Faculty of Health Sciences, Islamic university-Gaza, P.O Box, 108, Gaza, Palestine.

Abstract

The world is becoming increasingly under the threat of entering the "post-antibiotic era", an era in which the rate of death from common bacterial infections is higher than from cancer. *Pseudomonas aeruginosa* is an opportunistic multidrug-resistant pathogen and it is the main cause of morbidity and mortality in hospitalized patients and immunocompromised individuals. *P. aeruginosa* is considered as one of the most common pathogens, which can be highly difficult to cure its infection in human body. It is one of the major causes of nosocomial and ventilator-associated pneumonia characterized by high incidence and fatality rates. In addition, *P. aeruginosa* is commonly associated with chronic lung infections in individuals with cystic fibrosis. Thus, multidrug-resistant strains of *P. aeruginosa* contribute to high mortality in patients suffering of its infections.

One of the major virulence factors for this pathogen is its ability to form biofilms. This biological developed protects the pathogen from host immunity and contributes to its antimicrobial resistance. It is estimated that about 80% of infectious diseases are due to biofilm formation. Biofilm forming ability and antimicrobial resistance of this pathogen lead to many persistent and chronic bacterial infections.

Here, we review recent studies that are demonstrating how *P. aeruginosa* biofilm formation contributes to persistent chronic infection and its ability to resist antibiotic treatment. Thus, understanding the mechanisms of biofilm development of *P. aeruginosa* in association with resistance to antimicrobial agents is crucial to find way(s) for successful therapeutic interventions.

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Keywords
Biofilm; Bacterial Adhesion; *Pseudomonas Aeruginosa*; Antimicrobial Resistance; Quorum Sensing.

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Introduction

Pseudomonas aeruginosa is a Gram-negative bacillus, β-hemolytic, monotrichous bacterium with grape-like odor. It grows well at 25°C to 37°C, and its ability to grow at 42°C constitutes a distinguishing character from many other species. *P. aeruginosa* strains produce four types of pigments pyocyanin, pyoverdin, pyorubin, pyomelanin [1]. The extensive metabolic diversity and the flexibility of *Pseudomonas* spp. allow the organism to grow in a wide variety of environments, nutrient sources and to be an opportunistic pathogen. In particular, *P. aeruginosa* is able to form biofilms, develops antibiotic resistance, produces virulence factors, and rapidly evolves in the course of a chronic infection [1].

Role of *P. aeruginosa* virulence factors

Pseudomonas aeruginosa is a common health care associated pathogen. Numerous clinical conditions are highly associated with *P. aeruginosa* infection such as cystic fibrosis, burns, urinary catheterization, lumbar puncture and cancer chemotherapy [2].

P. aeruginosa has many virulence factors like hydrolytic enzymes, exotoxin A and endotoxin which are produced from the lipopolysaccharide component of its cell wall, phospholipases, exoproteases, phenazines, outer membrane vesicles; type III secreted effectors, flagella and pili [3]. These factors are contributing for the establishment of its infection. In addition, these factors can damage the epithelial cell lining, induce modifications in cell physiology, and function such as cell shape, membrane permeability and protein synthesis [4].

Previous work by Willcox in 2007 reported that *P. aeruginosa* coordinates the expression of virulence factors via quorum sensing [5]. It activates several pathways of the immune system e.g., it activates corneal epithelial cells receptors the toll like receptor. Based on activation, recognition of *P. aeruginosa* lipopolysaccharide or flagella and activation of the epithelial cells takes place, which leads to production of inflammatory mediators such as cytokines and chemokines. These are also recruiting polymorphonuclear leukocytes (PMNLs) to the site of infection to phagocytize and kill the *P. aeruginosa*. Nevertheless, continued recruitment and presence of these PMNLs in the corneal tissue and the production of proteases leads to destruction of corneal cells and tissue components. Moreover, it causes scarring and vision loss. It was concluded by Willcox that *P. aeruginosa* is considered as the most common cause of microbial keratitis [5].

The biofilm forming ability of *P. aeruginosa*, which is also considered as a virulence factor, contributes to the severity of cystic fibrosis (CF) disease [6]. Sagel, Gibson et al observed the increase in CF severity, lung inflammation and as a result, lung damage in patients infected with *P. aeruginosa* and *Staphylococcus aureus* [7]. A study showed that the presence of Gram-positive bacteria leads to up regulation of four molecules in *P. aeruginosa*, which are LasB elastase, rhamnolipids, exotoxins, and phenazines [8]. Development of biofilms can be associated with certain organisms everywhere in nature as well as in industrial and clinical environments such as dental plaques. More than 700 species of microorganisms were identified in extracellular polymeric substances (EPS) [9].

LasB elastase is a protease produced extracellularly by *P. aeruginosa*. It can digest the lung surfactant, the pulmonary antimicrobial enzyme lysozyme, and transferrin; additionally, it can hinder the ciliary movement [10]. Moreover, LasB protease interferes with macrophages engulfment and contributes to lung tissue injury, detraction of pulmonary action and allows the organism spreading to the bloodstream [11].

Rhamnolipids of *P. aeruginosa* are glycolipid biosurfactants. It solubilizes phospholipids so deterioration of lung surfactant activity occurs. It disrupts the PMNLs chemotaxis and macrophage function, and inhibits ciliary beating which leads to interference with airway immune response. Furthermore, rham-

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nolipids stimulate the airway epithelium to liberate IL-6 and IL-8 pro-inflammatory cytokines, which leads to inflammation [12].

The fourth molecule "phenazines", are pigments associated with cystic fibrosis disease development. The manufacturing of pyocyanin, which is the most dominant phenazine, participates in excess production of goblet cell, airway fibrosis and alveolar airspace devastation [11]. Pyocyanin production by *P. aeruginosa* disrupts the equilibrium of T helper signaling molecules leading to excess generation of IL-4 and IL-13. These two molecules are cytokines produced by Th2 and rise in the leakage of macrophage. The cytotoxic and immune modulatory effects of these cytokines lead to increased tissue damage and help *P. aeruginosa* survival. This immune alteration and elusion by many bacterial species may be the cause poor patient prognosis with infection by more than one species [8].

Increased virulence of *P. aeruginosa* in humans is accompanied by type three secretion (T3SS) expression [12]. These shoot exotoxins, macrophages and epithelial cells, causing excessive inflammatory response and associated tissue damage [10].

Pseudomonas aeruginosa is the most common cause of burns and intensive care units' infections [14]. Jackson, Lowbury *et al.*, reported that the colonization of burn sites by *P. aeruginosa* was responsible for graft failure, delayed healing and systemic complications [13]. *P. aeruginosa* burn infection may lead to an invasive infection and potentially fatal sepsis [15]. Therefore, it is essential to minimize the numbers of *Pseudomonas* in burns. Additionally, the new emergence of multidrug resistant strains of *P. aeruginosa* has further increased the urgency to discover more effective inhibitory agents [16].

Pseudomonas aeruginosa and Biofilm

Biofilm formation is a microbial complex mass in which cells are irreversibly attached to a substratum and embedded in a matrix of extracellular polysaccharide substances that they have produced. They can grow in and on living tissue, indwelling medical devices, industrial or portable water piping system and on solid surfaces [17].

Environmental signals stimulate switching of planktonic into biofilms which usually begins to form when a free-swimming bacteria attaches to a surface 18]. During this switch, many changes occur which include new phenotypic characteristics [18]. Also changes in gene expression [19] and genetic reprogramming that leads to a sessile lifestyle is the repression of flagella gene expression [19].

Some minerals like calcium, iron and copper can motivate biofilm formation and growth. When calcium concentration was increased to 80 ppm, the biofilm forming ability of *P. aeruginosa* was increased by 50% [20]. Components of Gram-negative bacteria, such as colanic acid alginate of *Escherichia coli*, glucose and mannose-rich components of *P. aeruginosa* play important roles in the biofilm formation. These byproducts construct and provide support for development of bacterial biofilms [19].

Biofilm matrix

The architectural structure and mechanical stability of the biofilms is due to the extracellular matrix [21]. Many factors infleunce the composition of biofilm matrices like the type of microorganisms and growth conditions. But in general, it consists of exopolysaccharides, proteins, and nucleic acids. Proteinaceous components include cell surface adhesins, protein subunits of flagella and pili, secreted extracellular proteins and proteins of outer membrane vesicles [22].

Every componenet in the matrix has significant function in the biofilm formulation. In 2006, a study demonstrated evidence that the absence of TasA or the exopoly-saccharide which are major components of *Bacillus subtilis* matrix led to complete failure to form complex multicellular communities [23].

Another study was conducted in 2019 showed that, stabilization of *S. aureus* biofilms are due to

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the positively charged cytoplasmic proteins that are liberated into the extracellular environment, where they make favorable electrostatic interactions with the negatively charged cell surface and extracellular DNA (eDNA) [24]. It was clarified that polysaccharide synthesis locus (Psl polysacaride) is a key element in EPS matrix of P. aeruginosa biofilm. It promotes cell–cell interactions and assembly of a matrix. Lec A and Lec B are produced by *P. aeruginosa* have affinities towards sugars pesent in the extracellular polymeric substance (EPS) matrix and contribute to the attachment of *P. aeruginosa* to specific host cells [25]. Binding of LecB to Psl participates in the retention of cells and EPS in a resurgent biofilm [26]. Chemical dissociation of Psl from the bacterial surface disrupted the Psl matrix as well as the biofilm structure [25].

Development of a biofilm

Microbial behavior has been well understood by research through the recognition of diverse characteristics of biofilms. Biofilm development on any surface includes five regular consecutive steps. Several bacterial and/or environmental factors influence the formation of biofilm, which is a dynamic and complex process [27].

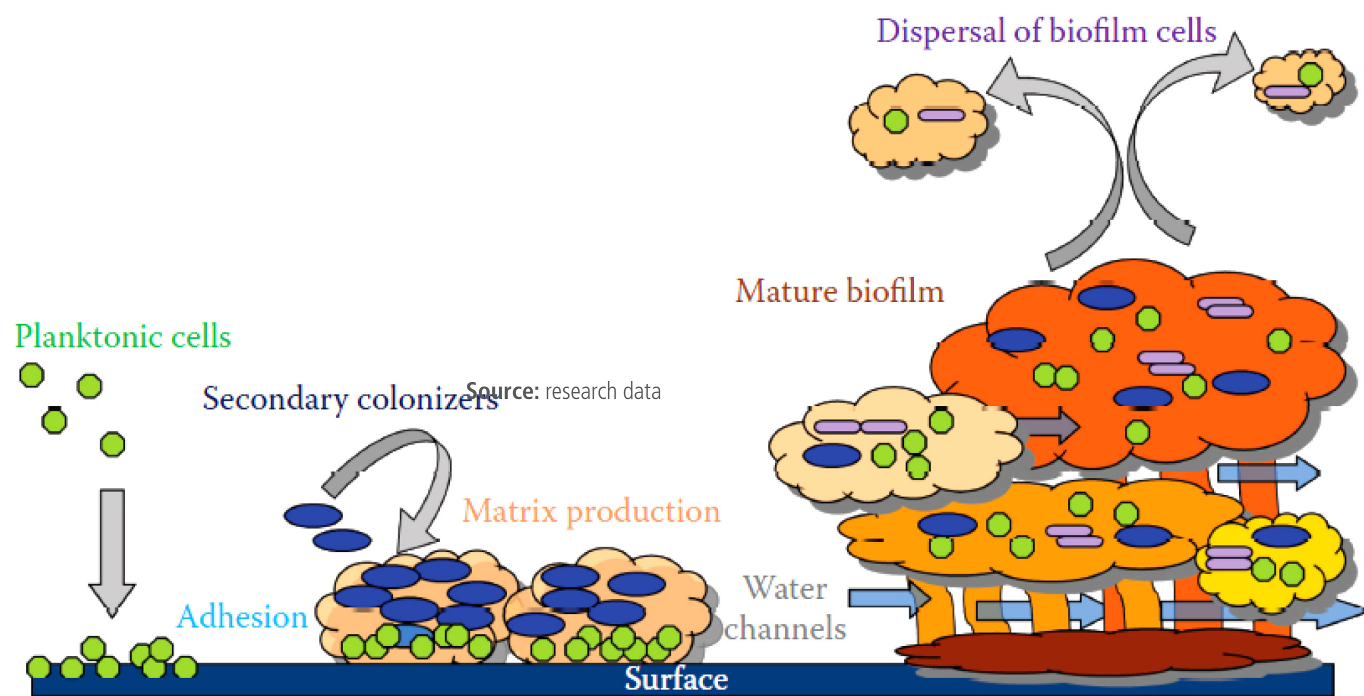
These environmental signals diverge among organisms. Some can form biofilms under any conditions that permit growth like *P. aeruginosa* and *P. fluorescens*. While others are producing biofilms only when minimal medium supplemented with amino acids like some strains of *Escherichia coli* K-12 and *Vibrio cholera*. Unlike *E. coli* O517:H7 which form a biofilm exclusively in media containing few nutrients [28]. Moreover, temperature, osmolality, pH, iron, and oxygen are other cues that can affect the biofilm formation beside the nutrient requirements [18].

The interaction between environmental and bacterial factors is important for the first step, primary reversible adhesion of the planktonic bacteria to the surface. This based on an equilibrium of attractive and repulsive power between the bacteria and the surface. Additionally, flagella-driven motility is a remarkable agent through the primary step of the biofilm production for example K12 strains of *E. coli* lacking flagella do not produce biofilms. Additionally, Chen and collaborators recently suggested that flagella-driven motility is also involved in biofilm formation of non-O157:H7 STEC [29]. The existence of surface installation like fimbrial adhesins influences the irreversible adhesion which is the second step in biofilm formation. For example, the attachment of shiga toxin producing *E. coli* (STEC) to surfaces conducted by many classes of fimbriae like type 1 fimbriae, type 4 pili, long polar fimbriae and F9 fimbriae [30].

The third step in biofilm formation is the development of micro colonies by the adhered microorganisms. The fourth step is excretion of extracellular polymeric substances (EPS) and development of a three-dimensional mature biofilm community. During biofilm maturation, bacteria continue to multiply and produce extracellular matrix. At this stage, the biofilm adopts a three-dimensional structure. This growth is mostly due to bacterium-bacterium cell interactions; several surface proteins and extracellular matrix components are involved in bacterial adhesion and biofilm architecture [31].

The final step in the biofilm formation cycle is its destruction, microbes detach and spread in to the environment to explore and colonize to new surfaces **(Figure 1)** [27]. Starvation is one possible signal for detachment; the details are not yet clear. (GA O'Toole, unpublished data) but Boyd & Chakrabarty clarified that the enzyme alginate lyase contribute in the detachment phase in *P. aeruginosa* and increased production of alginate lyase could accelerate dispersion and cell liberation from biofilms [32]. One study conducted by Allison and colleagues stated that loss of EPS leads to reduction in the biofilm of *P. fluorescens* after extended incubation [33].

Figure 1: Stages of biofilm formation [34].



Source: research data

Formation of EPS in which the microbial cells embedded is a prominent and unrivaled character of biofilms. Antimicrobial resistance is attributed to EPS; the scaffold for the biofilm [35].

The Formation of biofilms by *P. aeruginosa* and emergence of antimicrobial resistance

Biofilms have a detrimental action on human health. Bacterial biofilms can cause chronic infection(s) such as periodontal disease, chronic acne and osteomyelitis, and cystic fibrosis [36]. Biofilms are seen in patients infected with *P. aeruginosa*, medical implantation infected with *Staphylococcus aureus* or *Staphylococcus epidermids* [36].

Biofilms display complicated structure consists of pillar-like, mature macro colonies hedged by channels completed with fluid [37]. The unique asset of these macro colonies is their polymicrobial mix and their collaborative protective outcome(s) that diverse species of bacteria can provide to each other. For instance, antimicrobial resistant bacteria can secrete special enzymes or antimicrobial binding proteins that can shield adjacent non-antimicrobial resistant bacteria in a biofilm, at the same time it can transfer genes to other non-resistant bacteria to confer antimicrobial resistance, even between different species, horizontal gene exchange is greatly increased in biofilms [38]. Therefore, biofilms are linked to the emergence and spread of antibiotic resistant bacteria whereas horizontal gene transfer encourages evolution and genetic diversity of biofilms.

The highly polymicrobial populated biofilms, in comparison to a planktonic mode of growth, amplifies the total numbers of antimicrobial resistant mutants that can be chosen under antimicrobial pressure. Additional survival mechanism that numerous bacteria in biofilms have adapted is for a subpopulation to become metabolically inactive. Since antimicrobials act on metabolically active bacteria, the inactive bacteria in biofilms will remain unaffected by antimicrobials [38]. The EPS matrix restricts certain antimicrobial agents' diffusion from

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the surrounding into the biofilm. For example, it prevents the passage of certain classes of antibiotics especially those hydrophilic and positively charged ones, example; aminoglycosides. Therefore, several characteristics make biofilm formation is an essential tool and mechanism for antibiotic resistance, transfer of resistance plasmids and conjugative transposons, and a medium for intercellular communication (Quorum sensing) [38]. Thus, infection with multidrug resistant (MDR) strains of *P. aeruginosa* could present important threat to hospitalized patients in intensive care units [39]. MDR pumps play a role in biofilm resistance at low antimicrobial concentrations and there is a reason to believe that unknown MDR pumps might be overexpressed in *P. aeruginosa* biofilms [40].

Many factors help biofilm growth, for instance, increased level of mutations as well as with quorum-sensing-regulated mechanisms. Conventional resistance mechanisms such as chromosomal β-lactamase, up-regulated efflux pumps and mutations in antimicrobial target molecules in bacteria also contribute to the survival of biofilms [41]. Meanwhile, a study carried out by Haddadin *et al.* 2010 [42] showed a weak association between multiple drug resistance *P. aeruginosa* and strength of attachment to stainless steel surfaces. While an Indian study conducted on clinical isolates reported that 57% of biofilm producing *P. aeruginosa* were multidrug resistant (MDR). These isolates were resistant to aminoglycoside, beta-lactam group of antibiotics and fluoroquinolones, respectively. Whereas, non-biofilm producing *P. aeruginosa* showed 20% resistance to the same group of antibiotics [43].

A more recent study done by Redfern *et al.* 2020 [44], showed that the majority of MDR infections are caused by a slight number of "high-risk" clones that recently surfaced and spread worldwide. Using genome-wide and pan-genome wide association techniques, the study detected and verified potential essential genes involved in biofilm production and survival of *P. aeruginosa*.

Biofilms can be prevented by early aggressive antimicrobial prophylaxis or therapy and they can be treated by chronic suppressive therapy. The use of enzymes that can dissolve the biofilm matrix (e.g. DNase and alginate lyase) as well as quorum-sensing inhibitors that increase biofilm susceptibility to antimicrobials [45]. Thus, it could be generally concluded that biofilm is an important feature of virulence factors of any type of bacteria that can make a biofilm matrix, including *P. aeruginosa*.

Biofilm in human wounds

One to 2% of the population of developing countries has chronic wounds such as neuropathic foot ulcers, arterial ulcers, pressure injures and venous ulcers. It represents serious nuisance to patients because it is related to severe patient sufferance, depravation of job, decreased goodness of life and elevated expense to the health care system [46]. Wound infection lengthens the cure or recovery; treatment involves antibiotic management, elimination of necrotic tissues, suitable blood and oxygen supplying to the wound [47]. Recent studies show the presence of bacterial biofilms in chronic wounds [48]. This presence may be the cause of impaired and delayed wound healing [49] since leukocytes surrounding the biofilm prevent the healing of the wounds, and inflammatory cells produce proteases, which damage normal and healing tissues and immune cells [50]. *P. aeruginosa* in biofilms migrates via type IV pili and flagellum-mediated [51] and produces virulence factors that can hold back the action of host defense and protection systems [49]. It may explain the presence of these bacteria in the deeper regions of chronic wounds [52]. Wound healing influenced by many factors that can delay healing, but the diagnosis and management of wound infection is controversial and varies between clinicians.

Finally, an assessment of the factors affecting the progression from colonization to infection can help clinicians with the interpretation of clinical findings

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and microbiological investigations in patients with chronic wounds. An understanding of the physiology and interactions within multi-species biofilms may introduce more effective methods of treating infected and poorly healing wounds. Presence of consensus guidelines has helped to optimize clinical management [50].

Quorum sensing

Quorum sensing (QS) is a social behavior exhibited by bacterial strains whereby chemical-based communication modulates behavior of the aggregates of bacteria. It is the control of gene expression in response to cell density and it involves the production and detection of extracellular signaling molecules called auto inducers [53]. Accumulation of a minimal threshold stimulatory concentration of these auto inducers leads to the alteration of gene expression. By using these signal-response systems, bacteria concur specific behaviors on a population-wide scale and thus function as multicellular organism [54].

Researchers recognized many chemical classes of microbial signaling molecules produced by microbes **Table 1**. In general, Gram-negative bacteria use mostly N-acyl homoserine lacton (AHL) molecules (autoinducer-1, AI-1) while Gram-positive bacteria use primarily peptides (auto-inducer peptides, AIP or quorum sensing peptides) [55]. Communication is important for bacteria to organize several physiological activities such as symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation [56]. The main role of quorum sensing is to control the physiology of bacterial populations. This control is often done at the interface of different bacterial populations or at the bacterial-host margin [57].

Pseudomonas aeruginosa as a model of Quorum sensing

P. aeruginosa is an opportunistic pathogen, it uses a regulatory process called quorum sensing (QS) which organizes gene transcription according to cell density [63]. There are two major acyl-homoserine lactone (AHL) QS systems to *P. aeruginosa*: the LasR-LasI system and the RhlR-RhlI system. LasI and RhlI are signal synthases. LasI produces N-3-oxo-dodecanoyl-homoserine lactone (3OC12-HSL) and RhlI produces N-butanoyl-homoserine lactone (C4-HSL) [86-88]. The increase in cell densities leads to increase in the concentrations of these signals. The activation of transcription factors occurs in response to binding of 3OC12-HSL to LasR and of C4-HSL to RhlR. Because of activation of LasR and RhlR,

Table 1. Examples of bacterial quorum sensing systems and their controlled social traits.

Microorganisms	Average	Group-Derived Benefits	Reference
Bacillus subtlis	ComP/ComARap proteins	Competence, sporulation, biofilm formation, antimicrobial production,	[58]
Myxococcus xanthus	SasSRN	Fruiting body formation or sporulation	[57]
Pseudomonas aeruginosa	LasI/LasR RhlI/RhlR OscR (orphan)	Structured biofilm formation, virulence factors	[59]
Staphylococcus aureus	AgrC/AgrA	Biofilm formation, virulence factors	[60]
Streptococcus mutans	ComD/ComE ComR	Bacteriocins, biofilm formation, competence	[61]
Streptococcus pneumonia	ComD/ComE	Competence, fratricide, biofilm formation, virulence	[57]
Vibrio harveyi	LuxLM/LuxN LuxP/LuxQ	Bioluminescence emission, symbiosis	[62]

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the transcription of hundreds of genes is regulated. Also, QscR is a third counter regulatory AHL in *P. aeruginosa* [64]. It binds to 3OC12-HSL and to several other long-chain AHLs [65]. Improvement of LasR activation can be achieved by deletion of QscR [89-90]; it was detected by observing the earlier manufacturing of 3OC12-HSL and C4-HSL and pyocyanin [64].

A study demonstrated that QscR regulates 424 genes. This conclusion was reached by transcriptome analysis of a QscR-null mutant in comparison to the wild type [66]. QscR, which delay expression of LasR or RhlR-activated genes, is QS anti-activator in *P. aeruginosa* [67]. QteE and QslA are other anti-activator proteins that are not homologous to LasR or RhlR and do not bind AHL signals. They physically deactivate the ability of LasR and RhlR to induce gene transcription [67]. Several hypotheses have been in advance to detect the mechanism of QscR effects on the timing of AHL QS in *P. aeruginosa*. One study suggested that QscR alters the levels of lasI [64]. QscR could work through sequestering signal away from LasR. Ledgham *et al.* reported that overexpression in *E. coli* leads to the formation of inactive heterodimers with LasR by QscR which could postpone QS gene activation [68]. One recent study illustrated that there are four main pathways of QS dependent signaling present in P. aeruginosa. Each system consists of at least two major functional elements category of proteins [69]. It was concluded that *P. aeruginosa* QS system is complex due to its multiple inputs and transcription factors [70].

QS and antimicrobial resistance

The relationship between QS and antibiotic tolerance is multi-faceted because QS includes a universal alteration in bacterial gene expression and cell physiology. For instance, the supplement of AHLs to a logarithmic culture of *P. aeruginosa* was shown to elevate the number of persister cells in the population after treatment with carbenicillin and ciprofloxacin [71]. The presence of the *rhl*, and *las* QS systems in

P. aeruginosa are important for the biofilm production and their disturbance is associated with a higher sensitivity to the host immune system and antimicrobial compounds [72]. Moreover, *P. aeruginosa* has another QS system; the pqs system, which has been demonstrated to mediate a programmed cell death inducing extracellular DNA release, which promotes biofilm formation and antibiotic tolerance, benefitting the rest of the cell population [73]. The regulation effect of QS system on the expression of multidrug-resistant pumps is that both the expression of multidrug-resistant pumps can be regulated, and the QS system itself is also affected by the expression level of multidrug-resistant pumps. High expression of the efflux pump may promote the further activation of the QS system, promote the QS system's regulation of toxin infection factor synthesis and efflux pump expression, and enhance the infectivity and invasiveness of bacteria [73]. At the same time, researchers have found that the QS system regulates biofilm-forming genes and regulates bacterial resistance-related genes. In the presence of imipenem, the resistance gene *ampC* can be highly expressed in biofilms. This situation of high biofilm-forming bacteria, but low-expression suggests that the QS system may also regulate the expression of these resistance-related genes while regulating biofilm formation. This issue has attracted people's attention and may be another way available for the QS system to directly regulate the related genes in order to form drug resistance [74].

Mechanism of antibiotic resistance in bacterial biofilm

Antibiotic resistance is the microorganism's ability to resist the influences of drugs which can inhibit or kill their growth. For pathogenic bacteria, the dramatic increase in antimicrobial resistance in recent decades comprises a key threat to human health

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[75]. According to the CDC, the world is under a threat of entering the "post-antibiotic era", in which the rate of death from bacterial infections is higher than from cancer [76]. In the U.S. alone, 2 million infections and 23,000 deaths a year resulted from antibiotic-resistant bacterial infection and the cost of the resistance on economy is about $55–70 billion per year [77]. Biofilm forming ability of bacteria contributes to the antimicrobial resistance that leads to many persistent and chronic bacterial infections. Inside a biofilm, bacteria can attach to surfaces aggregate in a hydrated polymeric matrix of their own synthesis [78]. The biofilm's structural nature and sessile cells characteristics a protection environment against diverge conditions and the host´s defenses [79].

Conclusion

Despite decades of research, little is known about the molecular mechanisms of antibiotic resistance in biofilms [79]. However, a review article by Stewart and Costerton in 2014, illustrated that resistance and tolerance of biofilm may be due to several mechanisms [80]. These include slow penetration of antimicrobial agent through the biofilm, alterations in the biofilm chemical microenvironment (leading to zones of slow or no growth), response to adaptive stress [80-81]. Recently the article of Mittal proposed other mechanisms that have a role in antibiotic resistance. Poor penetration of antibiotics, exopolysaccharide, and degradation of environmental DNA (e DNA) in matrix are methods of struggling with antibiotic action. In addition, limited nutrient, slow growth and persistent cell formation make multilevel protections for antibiotic resistance. Genetically horizontal gene transfer and higher mutation frequency also display a crucial role in antimicrobial resistance in biofilm bacteria [82]. Although there are several excellent reviews on anti-microbial resistance in biofilms [82], many of them focus on a particular organism, a nominated mechanism or both. Moreover, it still is unclear whether the mechanisms discussed are more important in biofilms than in planktonic cells [80].

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