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UNDERSTANDING THE CONCEPT OF PSEUDOMONAS AERUGINOSA BASIC RESISTANCE MECHANISMS AND IMPACT ON CYSTIC FIBROSIS PATIENTS

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ABSTRACT

Numerous human illnesses, mostly connected to healthcare providers, are linked to Pseudomonas aeruginosa. It is linked to antibiotic resistance in hospitals, which makes treatment extremely difficult. However, biofilm-related P. aeruginosa infections provide one of the most difficult treatment problems. The intricate structure of the P. aeruginosa biofilm adds to the pathogenicity of this microbe by causing it to evade the immune system, cause persistent infections that are hard to treat, and result in treatment failure. We looked at a number of molecular facets of P. aeruginosa biofilm pathogenicity. It is believed that anaerobic circumstances, bacterial quorum-sensing systems, and environmental factors in the cystic fibrosis airway all contribute to the production of biofilms in the lung. In order to favor either acute infection or chronic colonization, P. aeruginosa has regulatory mechanisms that are sensitive to environmental signals. Respiratory tract-dwelling P. aeruginosa develop mutations that promote long-term colonization. P. aeruginosa biofilm development is changed by azithromycin, a macrolide that has therapeutic benefits for cystic fibrosis. Among the promising novel treatments that target the production of biofilms are compounds that interfere with quorum sensing.

Keywords: Pseudomonas Aeruginosa, Cystic Fibrosis Patients, Healthcare Providers

1. INTRODUCTION

Numerous infections linked to implants and tissues are caused by biofilms. Dental cavities, periodontitis, otitis media, persistent sinusitis, persistent wound alterations, musculoskeletal infections (osteomyelitis), biliary tract infections, bacterial prostatitis, native valve endocarditis, and infections linked to medical devices are among the infections linked to biofilms. Pseudomonas aeruginosa is the most researched microbe in relation to quorum sensing (QS) and is regarded as a model organism for researching biofilm development. The pathogenicity of the P. aeruginosa biofilm, along with its traits and QS features, are discussed in this

section. About 1 in 2500 people, mostly Caucasians of European ancestry, have cystic fibrosis, an autosomal recessive genetic condition. The gene was discovered in 1989 and encodes the cAMP-dependent chloride channel known as the cystic fibrosis transmembrane conductance regulator (CFTR), which is necessary for regular ion transport across epithelial cells. Even though children with cystic fibrosis have respiratory, gastrointestinal, and endocrine symptoms, the disease's most common cause of morbidity and death is the deterioration of pulmonary function. Airway surface liquid volume reduces and airway secretion viscosity rises when an abnormal CFTR impairs the capacity to control salt and chloride transport. Many bacterial infections develop colonization within this thick mucus. Anantharajah et al. (2016).

2. PSEUDOMONAS AERUGINOSA FEATURES

The rod-shaped, gram-negative aerobic bacteria Pseudomonas aeruginosa may be isolated from a variety of sources, including as soil, plants, and animal tissue Anantharajah et al. (2016). This bacterium uses its important binding elements, including flagella, pili, and biofilms, to thrive on water, various surfaces, and medical equipment. As a result, P. aeruginosa is prevalent in both natural and man-made settings, such as hospitals, lakes, and home washbasin drain Belaynehe et al. (2017). Human infections are caused by the opportunistic bacterium Pseudomonas aeruginosa. It is now a major contributor to antibiotic resistance and nosocomial infections Bleves et al. (2005). One of the opportunistic bacteria linked to infections in healthcare settings, such as ventilator-associated pneumonia (VAP), infections in intensive care units, bloodstream infections associated with central lines, surgical site infections, urinary tract infections, burn wound infections, keratitis, and otitis media, is Pseudomonas aeruginosa [Burns et al. (2001),Ciszek-Lenda et al. (2019)]. Pseudomonas aeruginosa is an organism that can produce a range of virulence factors, quickly acquire antibiotic resistance, and adapt to changes in its environment.

When insufficient treatment is utilized, P. aeruginosa infections can be fatal, especially when multidrug-resistant (MDR) strains are present Cobb et al. (2004). For the past 30 years, multidrug resistance has posed a risk to the health of both humans and animals. Additionally, P. aeruginosa is responsible for over 50% of healthcare-acquired infections, making it one of the most common pathogens in hospital settings. The fatality rates from P. aeruginosa are still high, ranging from 20 to 60 percent, despite the development of novel antimicrobial medications Cornelis et al. (2009).

Structural lung disorders, haematological neoplasms, transplantation, skin burns, recent antibiotic usage, implant presence, extended hospital stays, and mechanical ventilation are the main risk factors for P. aeruginosa infections Dauner & Skerra (2020). One of the most significant virulence factors in light of the pathophysiology of P. aeruginosa infections is the formation of biofilms. Being a well-known biofilm producer, Pseudomonas aeruginosa provides an intriguing in vitro model for studying biofilm development. Because of its heightened resistance to antibiotics, different irradiation treatments, environmental factors, disinfectants, and the immune system, Pseudomonas aeruginosa also colonizes a variety of surfaces, including medical supplies and equipment used in the food industry, and forms biofilms that result in chronic infections Dossel et al. (2012).

But according to recent research, more than 95% of kids have serologic or culture evidence of an intermittent P. aeruginosa infection by the time they are 3 years old Equi et al. (2006). Since early colonization with P. aeruginosa has been linked to worse clinical scores, lung function decline, and a worse chest radiograph in patients with cystic fibrosis when compared to age-matched cystic fibrosis patients who do not harbor P. aeruginosa, the fact that most children under the age of five have contracted the infection is concerning to the cystic fibrosis community. According to reports, children aged 8–13 who were colonized with P. aeruginosa before the age of 5 had a two to three times higher risk of dying Filloux & Vallet (2003).

In an effort to eradicate this organism, this has led many physicians to implement early intervention techniques. These tactics often entail the combination of an inhalation antibiotic with either intravenous antipseudomonal drugs or ciprofloxacin. Treatment duration varies greatly. Gambello et al. (1993) It has also been challenging to assess the results of early intervention because the outcome measures available to assess the effectiveness of this approach in young patients are extremely insensitive (e.g., lack of pulmonary function measures, noninvasive approaches to culture the lower respiratory tract, and chest radiographs).

2.1. THE CYSTIC FIBROSIS LUNG IS CONTINUOUSLY COLONIZED BY PSEUDOMONAS AERUGINOSA

The reason why P. aeruginosa so successfully targets the cystic fibrosis lung is unclear, despite the fact that persistent infection with the bacteria is clearly linked to pulmonary function losses and bad outcomes for people with the disease Gibson et al. (2003). Through an interaction mediated by asialo-GM1 binding, some scientists have observed that P. aeruginosa attaches to CFTR- airway epithelial cells more effectively than wild type cells; however, other authors have not reported similar findings. According to some researchers, CFTR is a P. aeruginosa lipopolysaccharide receptor Gillis (2005). Lung epithelial cells missing CFTR internalize P. aeruginosa less effectively than wild type control cells, according to in vitro research; sloughing and cleaning of bacterium-laden epithelial cells may help remove germs from the airway. Additionally, cytotoxins like pyocyanin, which are produced by P. aeruginosa, have been shown to change CFTR expression and trafficking to the surface of epithelial cells Goltermann & Tolker-Nielsen (2017). The high colonization rates of P. aeruginosa in other chronic obstructive lung illnesses when CFTR is not altered may be explained by this. P. aeruginosa can adapt and flourish in the pulmonary environment thanks to a variety of regulatory mechanisms and virulence factors, as will be covered below.

2.2. PSEUDOMONAS AERUGINOSA EVASION OF CYSTIC FIBROSIS HOST IMMUNE RESPONSE

P. aeruginosa often takes on a planktonic lifestyle during acute infections or a sessile lifestyle during chronic infections. Pseudomonas aeruginosa encodes virulence proteins that improve its fitness and chances of surviving in a human host, among other virulence mechanisms (Table 1). By causing severe wounds, tissue necrosis, evasion, and immune system damage, these virulence factors let bacteria proliferate and survive while navigating the host's cellular machinery Guo et al. (2014).

Table 1

Table 1 Main Virulence Mechanisms P. Aeruginosa in Cystic Fibrosis Patients		
Antibiotic resistance	Efflux pumps	
	Modifying enzymes	
Motility	Flagella	
	Type IV pili	
Biofilm structure & dynamics	Rhamnolipids	
	alginate	
Iron scavenging	Proteases	
	siderophores	Pyochelin
		pyoverdine
Cytotoxicity	pyocyanin	
	T3SS	
	Endotoxin A	
	HCN	
Immune evasion	elastase	Alkaline protease

1) Pseudomonas aeruginosa Biofilms in cystic fibrosis

P. aeruginosa may adopt two different lifestyles: planktonic and sessile cells. While P. aeruginosa may form sticky clusters in permanent rearrangements on natural or synthetic surfaces, which are characterized by the release of an adhesive and protective matrix, the planktonic state can be found in a liquid culture suspension Halldorsson et al. (2010). This bacterial population sticking to a surface is known as a "biofilm," and it seems to be an adaptive reaction to an environment that is more or less unsuitable for planktonic growth Hendrie (1989).

It has been assessed if bacterial biofilms display phenotypic variety as a result of internal chemical variation, such as pH shifts, oxygen gradients, and nutritional variations. Even in a genetically uniform population, individual bacterial cells are able to sense these environmental variables, which results in metabolic activity and differential gene expression Hybiske et al. (2004). Compared to planktonic cells, biofilms are around 10–1000 times more resistant to antibiotics. This is caused by changes in protein synthesis and metabolic activity, as well as the inability of antibiotics to penetrate the intricate polysaccharide matrix Iiyama et al. (2017).

Taking into account all of these factors, it is very hard to eradicate infections brought on by biofilm-forming P. aeruginosa, such as those in cystic fibrosis, and treating infections brought on by MDR strains presents further difficulties Jacobsen et al. (2020). Patient morbidity and mortality are raised by these issues. Other consequences include increased rates and length of hospitalization, as well as higher treatment costs Kalluf et al. (2017). Even in the absence of direct interaction with bacteria, macrophages exposed to biofilms develop into cells that cause tissue damage due to their secretory characteristics Kang et al. (2003).

The formulation of methods to prevent, control, and eliminate biofilm-associated illnesses requires a better knowledge of the composition and structure of the biofilm, including its molecular processes and antimicrobial tolerance.

Additional proof that biofilm development takes place in vivo is provided by the chemicals produced by P. aeruginosa residing in biofilms in cystic fibrosis sputum. For instance, P. aeruginosa has many quorum-sensing mechanisms that generate and detect N-acylhomoserinelactones, which are tiny molecules that permeate bacterial membranes and build up in the surroundings Kong et al. (2006).

These signaling molecules become abundant as the number of bacteria rises, activating gene sets, including those necessary for the formation of biofilms. Rhamnolipids are one type of virulence factor that is directly regulated by quorum sensing molecules. Sputum from patients with cystic fibrosis has incredibly high levels of rhamnolipids, which are amphipathic compounds produced by bacteria in biofilms Kownatzki et al. (1987). Increased transcription of rhamnolipid genes during growth in cystic fibrosis sputum was corroborated by microarray tests analyzing laboratory strains Lee et al. (2005). It is unknown what part of the sputum from cystic fibrosis the bacteria recognize and use to produce more rhamnolipids.

There is ongoing discussion and investigation about the significance of biofilm formation for P. aeruginosa chronic colonization in cystic fibrosis. The capacity of strains that were successively isolated from individuals with cystic fibrosis over a long period of time to produce biofilms in vitro was investigated by Lee et al. Li et al. (2015). With a tendency towards reduced adhesion and biofilm development in late isolates, these scientists discovered notable variation in biofilm formation. They came to the conclusion that biofilm development, as assessed in vitro, might not play as significant a role in P. aeruginosa persistence over the long term as previously believed. A disclaimer about the aforementioned study is that aerobic, not anaerobic, conditions were used to detect P. aeruginosa biofilm development.

A- Biofilm Structure and physical condition

One of the main constituents of the biofilm in mucoid strains of P. aeruginosa is alginate, an exopolysaccharide. This molecule's high molecular weight is made up of O-acetylated, 1-4 linked D-mannuronic and L-guluronic acids. AlgT-factor controls the expression of these enzymes, which are encoded by the algD operon for alginate production Li & Lee (2019). Mucoid P. aeruginosa strains overproduce alginate, which is crucial for the maturation and durability of biofilms even though it is not required for their formation Lister et al. (2009). As an adhesin, alginate binds to respiratory tract mucus. Its acetyl groups make the biofilm more viscous, which causes water and nutrients to build up. By shielding P. aeruginosa from phagocytosis and scavenging reactive oxygen species generated by activated macrophages, alginate also helps the bacteria remain viable Mall et al. (2004). Lung inflammation is also exacerbated by alginate's high leukocyte response and radical oxygen species emission. Alginate's capacity to bind aminoglycoside antibiotics and prevent their entry into the biofilm is another significant characteristic that increases antibiotic resistance and clinical treatment failure Mann & Wozniak (2012).

The extracellular milieu contains amphipathic molecules called rhamnolipids. They are secondary metabolites made up of rhamnose and O-glycosidic connected by a dimer of a -hydroxy fatty acid linkage. These chemicals can break down the lung surfactant, which lowers the transepithelial electrical resistance and causes the respiratory cells' tight connections to break McKnight et al. (2000).

This disease increases the risk of pneumonia by encouraging P. aeruginosa to colonize the airways. Rhamnolipids maintain the non-colonized channels open, which aids in the design of biofilms. Because of their surfactant qualities, rhamnolipids reduce surface tension and promote sliding motility in the absence of flagella. Rhamnolipids are produced in iron-restricted circumstances, which enhance twitching motility. Blocking flagellin-induced human defensin 2 can reduce host innate immunity Michalska (2015).

B- Environmental impact on pseudomonas aeruginosa biofilm formation in cystic fibrosis patients

P. aeruginosa produces acute pneumonia in susceptible hosts in addition to chronic colonization. However, the virulence factors produced during chronic persistence are different from those present during acute infection Nguyen (2006). For instance, P. aeruginosa may use a device like a needle called the type III secretion system (TTSS) to inject deadly effector chemicals straight into epithelial cells during an acute infection. Bacteria suppress this mechanism when they develop biofilms and colonize an area over time. The low oxygen level of the airway surface fluids in the lung of a person with cystic fibrosis is one of its characteristics. As a result, researchers have looked at the anaerobic biofilm production of P. aeruginosa. Different genes are needed for anaerobic vs aerobic biofilm development, even though P. aeruginosa may produce biofilms at low oxygen tension.

The presence of thick mucus is a second feature of the pulmonary environment in cystic fibrosis. The biomass of biofilms produced on a mucin-coated surface was found to be higher than that of biofilms grown on glass, actin, or DNA substrates. The biofilm's architecture also varied, with the majority of the cells grouped together in huge clumps. Tobramycin resistance was higher in these mucin-associated biofilms, presumably as a result of structural modifications that decreased antibiotic penetration.

High levels of the pro-inflammatory cytokine IL-8 and a severe neutrophil inflammatory infiltration are two other features of cystic fibrosis airways. Walker et al. observed thicker P. aeruginosa biofilms when neutrophils were present than when they weren't in their investigations of the bacteria's biofilm development. Additionally, lysed neutrophils encouraged the development of biofilms on actin and DNA-containing filaments, which are substances seen in sputum from cystic fibrosis. The scientists came to the conclusion that P. aeruginosa survival in the face of a strong inflammatory response may be enhanced by the biofilm-forming potential of dying neutrophils in the cystic fibrosis lung Ochsner & Reiser (1995).

C- Regulation Systems Involved in Pseudomonas aeruginosa Biofilm Formation

Las, Rhl, Pqs, and Iqs are the names of the four most significant interrelated QS systems in P. aeruginosa. Crosstalk between several cellular signals is made possible by these hierarchical network architectures via QS. Through 3-oxo-C12-HSL and C4-HSL, respectively, the LasR/LasI and RhlR/RhlI QS systems control synthesis and signal transduction. 3-oxoC12-HSL, which is produced by LasI, activates the cytoplasmic receptor. For the creation of biofilms, LasR controls the expression of genes that produce exotoxins A, hemolysins, proteases, and elastases. It was discovered that LasR in P. aeruginosa plays a crucial role in controlling the expression of the lasB gene, which codes for the metalloprotease elastase. Later, it was discovered that LasR was required for the transcription of toxA, lasA, and aprA. It is therefore regarded as a worldwide regulator of P. aeruginosa's virulence genes Orgad et al. (2011).

N-(3-oxododecanoyl)-homoserine lactone (OdDHL) was recently identified as the chemical structure of this gene-mediated Pseudomonas autoinducer (PAI).

The LasR protein is necessary for P. aeruginosa to activate lasB and a number of other virulence genes. The action of LasR depends on the PAI that the bacteria create.

They have found another autoinducer, which has been structurally characterized as N-butyrylhomoserine lactone (BHL). BHL does not directly interact

with the LasR protein to trigger the expression of the lasB gene. The cognate receptor of BHL is RhlR, a regulatory protein encoded by the rhamnolipid synthase gene cluster rhlABR Pier (2000).

OdDHL and BHL are unable to replicate the third QS signal, 2-heptyl-3-hydroxy-4-quinolone, commonly known as Pseudomonas quinolone signal (PQS), which is linked to lasB expression in a lasR mutant of P. aeruginosa.

A novel family of QS signal molecules includes the fourth intercellular communication signal, or Iqs. AmbBCDE is a cluster of non-ribosomal peptide synthase genes that includes the genes involved in Iqs synthesis. It results in a reduction in both PQS and BHL signal generation when it is disturbed. The expression of virulence components including elastase, rhamnolipids, and pyocyanin is induced via the same mechanism.

P. aeruginosa's QS circuits are intricate. OdDHL activates the Las system, which in turn stimulates the multimerization of the LasR-OdDHL complex and, in turn, the transcription of rhlR, rhlI, lasI, and other virulence genes. Additionally, the RhlR-BHL complex forms a second positive feedback loop by activating the expression of RhlI and its own regulon. Additionally, PqsR, the transcriptional regulator of the PQS biosynthetic operon pqsABCD, is favorably regulated by LasR-OdDHL. In turn, it was discovered that PQS might increase RhlI transcription, which would affect BHL synthesis, the general expression of the Rhl QS system, and indirectly modify the phenotypes that depend on Rhl Remold et al. (n.d) .

Pseudomonas aeruginosa passively infiltrates hosts with weakened epithelial barriers or deficiencies in the local or systemic immune systems. Nevertheless, the Rhl QS system can be activated by an outer-membrane protein called OprF that can identify and bind to IFN-γ. The Rhl QS system is activated by this route tein, OprF, which may identify and bind to IFN-γ. This route increases lecA expression and the synthesis of galactophiliclectin, the virulence protein that is encoded by it. Another virulence factor that is elevated when IFN-γ is present is pyocyanin. Human dynorphin is an endogenous Ƙ-receptor agonist that has the ability to pass across bacterial membranes and may trigger PqsR and PqsABCDE expression, which would increase PQS and HHQ 71 production Reynolds & Kollef, M. (2021).

Endothelial cells generate C-type natriuretic peptide (CNP), which is also important in controlling P. aeruginosa QS by raising intracellular cAMP concentrations, which triggers the activation of the global virulence activator Vfr.

P. aeruginosa's biofilm development is regulated by the stationary-phase sigma factor RpoS. RpoS directly or indirectly regulates over 700 genes, the majority of which are connected to QS. The HsbR-HsbA partner-switch has been shown to be essential for the post-translational control of RpoS. The FlgM-HsbA partner-switch is linked to this mechanism, which causes RpoS to be released or sequestered. This process is probably the most effective one the bacteria has discovered for choosing to swim or to create and spread its biofilm.

PQS has more recently been linked to the initial phases of biofilm formation.

It participates at several levels and acts as an outer membrane vesicle (OMV) inducer. Effective biofilm dispersion is contingent upon the creation of PQS-induced OMVs, as evidenced by the differential production of PQS and OMVs during P. aeruginosa biofilm growth. Interestingly, the majority of the physiological processes and virulence phenotypes of P. aeruginosa are accounted for by these QS-related genes, which make up around 10% of the genome.

D- Antibiotics change how Pseudomonas aeruginosa forms biofilms

A cornerstone of all treatment plans for people with cystic fibrosis is antimicrobial medication targeted against P. aeruginosa. Unfortunately, P. aeruginosa uses a number of mechanisms, including as the creation of b-lactamases and drug efflux pumps, to make it resistant to several kinds of antibiotics. By physically preventing antibiotic penetration, biofilms may also boost resistance Ryder et al. (2007)

According to a recent study, P. aeruginosa biofilm development is facilitated by sub-therapeutic aminoglycoside concentrations through the expression of a protein known as Arr (aminoglycoside response regulator). 13 out of the 14 cystic fibrosis isolates analysed in this study developed biofilms as a result of tobramycin; the strain that did not react lacked the arr gene.

There might be significant therapeutic ramifications to this discovery. Most cystic fibrosis treatment plans include inhaled aminoglycosides, which can expose patients to subtherapeutic antibiotic doses. A phosphodiesterase called Arr is involved in the metabolism of the tiny second messenger dicyclic GMP (dicGMP). Antibiotic resistance, virulence factor expression, and biofilm formation in P. aeruginosa depend on a number of genes encoding proteins thought to be involved in di-cGMP metabolism. Di-cGMP signalling has been suggested as a potential target for antimicrobial treatment since it seems to be common among prokaryotes.

According to several recent research, including prospective double-blind placebo-controlled trials, people with cystic fibrosis who regularly take the macrolide azithromycin experience fewer pulmonary exacerbations. 185 individuals were randomly assigned to receive either azithromycin or a placebo by Saiman et al. Saiman & Marshall (2003). Five months later, the therapy group had improved forced expiratory volume, gained more weight, and experienced fewer pulmonary exacerbations. However, it is still unknown how azithromycin works. Additionally, it was discovered that azithromycin had no effect on P. aeruginosa adhesion to epithelial cells, CFTR expression, or epithelial ion transport.

Azithromycin seems to reduce quorum-sensing activity and postpone the start of biofilm formation. Remarkably, azithromycin causes a significant upregulation of genes linked to acute infection, such as the TTSS. However, it is unclear if the circumstances employed in these in vitro tests are representative of the environment in vivo. According to other research, azithromycin does prevent P. aeruginosa lab strains from synthesizing proteins and reduces the expression of genes necessary for the production of biofilms. Clinical improvement was observed in individuals taking azithromycin regardless of whether they had P. aeruginosa colonization, according to a subset analysis of a recent trial of children receiving azithromycin or a placebo Schuster & Greenberg (2006). Despite the possibility that P. aeruginosa was present but not recovered in culture, this finding implies that in-vivo azithromycin has an anti-inflammatory impact rather than a particular antipseudomonal effect.

2) Motility (Flagella and Type IV Pili)

Flagella are essential for P. aeruginosa initial attachment to surfaces. Twitching motility has been necessary for normal biofilm development after microcolony formation. The flagellum is also responsible for P. aeruginosa swimming motility in low-viscosity environments.

Rotation drives this action, creating a force that propels the bacteria along. While the motility mechanism is linked to dispersal in the latter stages of biofilm formation, flagellar attachment plays a significant role in the beginning stages of

biofilm formation. Appropriate timing of motility control is necessary for robust biofilms during the maturation period. Type IV pili, which are crucial for mediating adhesion to mucosal surfaces and subsequent colonisation, are necessary for twisting motility. The flagellum independent form of surface motility is associated with the retraction and extension of type IV pili, which push the cells along the surfaces. Additionally, these pili are filamentous, hair-like appendages that are polarly situated. The cytoplasmic motor (PilBTUCD), the inner membrane alignment (PilMNOP), and the OM secretin pore (PilQF) are the three subcomplexes that make up the structure of pili Smith (2003).

Pili are thought to be essential components for the initiation of infections because they mediate adhesion and motility. Furthermore, these components regulate twitching motility, which is employed to quickly colonize various surfaces. This suggests that the force propelling the bacterial cell forward is produced by several cycles of T4P fiber extension, adhesion, and retraction. Two cytoplasmic membrane-associated ATPases, which polymerise and depolymerize PilA, respectively, are responsible for the pilus movement. Furthermore, the binding of pili tips to DNA is likely linked to biofilm development and natural transformation Spoering & Lewis (2001).

3) Immune Evasion (Elastase and Alkaline Protease)

Extracellular proteases linked to crucial invasion in acute P. aeruginosa infections include LasA and LasB elastases, type IV protease (PIV), P. aeruginosa small protease (PASP), Large ExoProtease A (LepA), alkaline protease (AprA), P. aeruginosa aminopeptidase (PAAP), and MucD. T2SS, which is controlled by QS systems, secretes LasB and LasA elastases, which break down host elastin [46]. The lasB gene encodes the most prevalent protease, LasB elastase, a metalloprotease and the primary extracellular virulence factor. LasA expression has recently been linked to antibiotic resistance in P. aeruginosa clinical isolates.

Phagocytic evasion is made possible by alkaline protease, also known as aeruginolysin in P. aeruginosa, another metalloendopeptidase generated by T1SS (aprA gene encoded), which disrupts endothelial components (fibronectin and laminin) and breaks down complement proteins (C1q, C2, and C3) and cytokines (IFN, TNF, and IL-6). Additionally, it contributes to the synthesis of pyocyanin (and other virulence factors) and cleaves free flagellin monomers, which decreases the mucociliary clearance of germs by activating epithelial sodium channels Stewart & Costerton (2001).

To increase survival in the cystic fibrosis lung, P. aeruginosa uses a variety of tactics in addition to biofilm production. To evade the innate immune system's identification, P. aeruginosa can stop producing highly immunogenic chemicals. The flagellin expression is a well-researched illustration of this. For swimming motility and initial adhesion during biofilm development, P. aeruginosa needs its solitary, unipolar flagellum. Flagellin uses Toll-like receptor 5 to strongly stimulate the host immune response Stewart & Franklin (2008). Many genes that encode pro-inflammatory mediators like IL-8 are transcriptionally induced when flagellin is added to human airway epithelial cells. When P. aeruginosa is exposed to either normal or cystic fibrosis sputum, it rapidly suppresses flagellin transcription and expression. It is believed that this reaction aids P. aeruginosa in avoiding immune system identification. According to a recent study, the molecule that suppresses the production of flagellin is neutrophil elastase. Swimming motility of bacterial isolates decreases with time, most likely as a result of flagella loss, according to motility studies of P. aeruginosa obtained from the same cystic fibrosis patient during a 20-year period Stover et al. (2000).

Prolonged lung colonization also causes P. aeruginosa isolates to change into a mucoid phenotype. The exopolysaccharide alginate, a linear copolymer of b-1,4-linked D-mannuronic acid and a-L-guluronic acid, is produced in significant quantities by mucoid P. aeruginosa. Mutations that cause loss of function in mucA, a negative regulator of alginate production, are typically the cause of mucoid phenotypes in North America. Researchers have hypothesised that the pulmonary environment benefits from alginate formation. Alginate synthesis may assist P. aeruginosa evade identification by the host immune system in vivo, as evidenced by the reduced inflammatory response displayed by airway epithelial cells exposed to alginate Tam et al. (2010).

4) Antibiotic Resistance (Pump Efflux and Modifying Enzymes)

When β-lactams, such as piperacillin, ceftazidime, cefepime, ceftolozane, carbapenem, and others, are used as first-line treatments, the formation of lactamases has been the most significant cause of antimicrobial resistance. Cephalosporinases, ampC hyper-expression, extended-spectrum β-lactamases, and carbapenemases are the most significant β-lactamases found in P. aeruginosa. The main obstacle at the moment is carbapenemases, which limits the range of available treatments to a small number of medications, including outdated and harmful medications like aminoglycosides and polymyxins Thirumalmuthu et al. (2019).

One of the best-described resistance aspects is the efflux pump systems, which are in charge of removing antibiotics from the cell. The fact that drug-resistant P. aeruginosa has spread throughout the world should not be overlooked. According to reports, efflux pumps work in tandem with bacterial biofilms and outer membrane barriers to provide the maximum level of resistance. Efflux transporters are linked to the primary source of antibiotic multidrug resistance in this mechanism Tuon et al. (2012).

Enzymatic changes of aminoglycosides are the cause of the high degree of resistance found in many bacterial species. Aminoglycoside-modifying enzyme (AME)-encoding genes are among the significant resistance genes that are transferable between Gram-negative bacteria, primarily in P. aeruginosa. Aminoglycosides are thought to be the most effective medications for treating P. aeruginosa infections that pose a serious risk to life. Nonetheless, one of the most significant processes that results in resistance to them is enzymatic modification Tuon et al. (2019).

AMEs are categorised as acetyltransferases (AACs), nucleotidyltransferases (ANTs), and phosphotransferases (APHs). These enzymes cause the aminoglycosides to undergo N-acetylation, O-nucleotidylation, and/or O-phosphorylation, respectively, which renders medications inactive and renders treatment useless. Furthermore, transposons, integrons, and plasmids are examples of mobile genetic components that can help clinically significant genes spread quickly. Additionally, AME genes were identified by one group as the primary source of aminoglycoside resistance in MDR isolates of P. aeruginosa van et al. (2005).

5) Cytotoxicity (HCN, Exotoxin A, T3SS, and Pyocyanin)

HCN is only synthesised by a few number of bacterial species, including P. aeruginosa. Numerous physiological functions can be inhibited by the secondary metabolite cyanide; HCN has been shown to impede aerobic respiration. HCN may be discovered in sputum samples from people with cystic fibrosis who have contracted P. aeruginosa, as well as in the headspace of in vitro P. aeruginosa cells. The majority of the isolates classify exotoxin A as the primary toxigenic virulence factor of P. aeruginosa that is exocyted by T2SS. It is separated into one subdomain

and three structural domains. Antiparallel strands make up the majority of the N-terminal domain (I), which is in charge of attaching to host cells; six helices with membrane-translocating activity make up the middle domain (II); and the poisonous moiety is found in the C-terminal domain (III) Vu et al. (2009).

Several proteins make up the P. aeruginosa T3SS, which injects toxic effectors into the cytosol of the host cell (injectisome). These proteins include (i) a secretion system, which delivers effectors across membranes, and (ii) a translocation apparatus, which transfers the effectors across human cell membranes. The secretion apparatus consists of an oligomerized secretin ring at the OM (PscC), an IM lipoprotein ring (PscJ), a cytoplasmic ATPase (PscN), and a helically polymerised protein (PscF). The translocation machinery is understood by two hydrophobic proteins (PopB and PopD), which interact with the membrane of human cells.

They create a translocation pore and the hydrophilic protein PcrV, which are essential for PopB and PopD to properly assemble and enter onto the surface of human cells. Although T3SS is not necessary for infection, it intensifies the infection by allowing P. aeruginosa to damage the epithelium, spread throughout the bloodstream, and inhibit host innate immune responses (either through a dependent or effector-independent mechanism) Walker et al. (2005).

6) Iron Scavenging (Proteases and Siderophores)

Pyoverdine (PVD) is a peptide, whereas pyochelin is a salicylate-based siderophore with a low iron affinity West et al. (2002). Energy is used in the PVD synthesis process in P. aeruginosa, which first makes pyochelin and only transitions to PVD production when the iron content drops significantly. A binding iron-conserved dihydroxyquinoline chromophore is a component of PVD. Although over fifty pyoverdines are reportedly created, they are divided into three categories (PVDI, PVDII, and PVDIII) according to the variance in the peptide chain. Iron from human proteins is scavenged by one of the pyoverdines (PVD). carried out by a network of membranes, periplasmic space transporters, and efflux pumps. Despite being necessary, excessive iron concentrations cause reactive oxygen species, which can be harmful to cells. Therefore, when there is enough intracellular iron, the ferric uptake regulator, also known as Fur, turns off this mechanism [58]. Since the ECM retains the iron that is sequestered inside the three EPSs, it is crucial for maintaining equilibrium in biofilms (29948830). ETA, an endoproteinase (PrpL), and PVD itself are all produced by PVD acting as a signaling molecule.

3. CONCLUSION

P. aeruginosa biofilms' pathogenicity mechanisms, including their traits and QS features, are incredibly intricate. Despite the apparent simplicity of a biofilm's structure, the genes and processes that contribute to its creation are varied and challenging to understand. Additionally, the majority of research done to find them has been done in vitro, which is not how human infections actually occur. One way to deal with this would be to analyse clinical samples using transcriptomics and metabolomics. However, because the majority of these infections are polymicrobial and include local microbiota, it would be challenging to distinguish the data relating to P. aeruginosa from those of other bacteria.

The understanding of P. aeruginosa biofilm production in vitro has advanced significantly. The behavior of clinical isolates in the lung of patients with cystic fibrosis must yet be reconciled with these in vitro laboratory findings. Researchers are still working to create mice models that more closely resemble the pathophysiology of chronic P. aeruginosa pulmonary infections and cystic fibrosis

lung disease Zaborina et al. (2007). Meanwhile, advancements in technology enable researchers to work towards the objective of comprehending the behavior of P. aeruginosa in human cystic fibrosis. Clarifying how P. aeruginosa interacts with the host is crucial for the advancement of novel treatment development.

CONFLICT OF INTERESTS

None.

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