Journal of Population Therapeutics & Clinical Pharmacology

A REVIEW PAPER: ON VIRULENT DETERMINANTS AND ANTIMICROBIAL RESISTANCE MECHANISMS OF *PSEUDOMONAS AERUGINOSA*

Mehraj Gul[1], Fawad Shabir Memon[1], Ahad Mehmood[2], Waseem Ullah Khan[1], Sarfaraz Ali[1], Abdul Latif[3]

[1]Quality Control Laboratory, National Institute of Health Islamabad, Pakistan
[2]Department of Microbiology, Abbottabad University of Science and Technology
[3]University of Balochistan, Quetta, Pakistan

Corresponding author: Mehraj Gul, email. m.gul@nih.org.pk

Abstract: *Pseudomonas aeruginosa* is gram negative, rod shaped, ubiquitous and an opportunistic pathogen that displays various virulent determinants and possess different antimicrobial resistance mechanisms. It can thrive in different ecological niches and responsible for various nosocomial infections e.g. wound, UTIs and respiratory infections. According to the WHO it is placed in list of most critical priorities in need of drug development due its increased intrinsic & acquired antimicrobial resistance mechanisms and its ability to bear different biocides e.g. disinfectants, antiseptics and preservatives. The major virulent determinants of *P. aeruginosa* are Fimbriae, Polar flagella, secretion systems (Type I–VI), some enzymes Elastase, protease, hemolysin and some quorum-sensing molecules. The lipopolysaccharide (LPS) and exopolysaccharides such as alginate, pel & psl are also major contributors in increased resistance against commonly prescribed antibiotics and biocides. The other Major resistance mechanisms in *Pseudomonas aeruginosa* are Impermeability of membrane, Biofilm formation, Efflux systems and Inactivation & structural modifications in antibiotics.

Conclusion: Understanding and exploring the virulent determinants and resistance mechanisms shed light on formidable challenges posed by *Pseudomonas aeruginosa*. Understanding these complex phenomena's in *Pseudomonas aeruginosa* play a crucial role in public health, infection control and development of effective & novel therapeutic strategies. The genetic and molecular basis of these resistance mechanisms can still be major targets for developing novel therapeutic agents. Significant research has been carried to cope with the expounding resistance mechanisms of *Pseudomonas aeruginosa* but much remains to be discovered. It still remains a top priority for the researchers and scientists to stay ahead of this versatile pathogen's evolutionary strategies. In order to safe guard the well-being of patients collaborative efforts are required among researchers, scientists, clinicians and policymakers to fight against the threats posed by *Pseudomonas aeruginosa.*

Key words: Opportunistic pathogen, virulent determinants, Antimicrobial resistance AMR, biocides, secretory systems, exopolysaccharides, biofilm, efflux system, therapeutic strategies.

Introduction

The antimicrobial resistance (AMR) and infectious diseases are global public health issue. The most life-threatening infectious bacterial pathogens are named as "ESKAPE" that is an acronym for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. These life threatening bacterial pathogens possess great ability to resist against multiple antimicrobials (Pachori et al., 2019). In consequence, the World Health Organization placed these multidrug resistant bacterial pathogens such as *Pseudomonas aeruginosa* in the list of critical priority pathogens for research and development of new antibiotics (World Health Organization 2017). *Pseudomonas aeruginosa* is a bacterium which is present everywhere in air, water & soil and it causes different nosocomial infections such as urinary track, wound and respiratory infections which are very difficult to treat (Snyder et al., 2013: Kumarage et al., 2019: Slekovec et al., 2012: Quick et al., 2014). It is also responsible for high mortality in cystic fibrosis patients (Parkins et al., 2018). It has the ability to switch into biofilm lifestyle, remains for years in the lungs of cystic fibrosis patients is due to its high adaptive qualities and also remains protected from immune cell and tolerates antibiotics (Høiby, 2011). It is a robust microorganism with high adaptive qualities to grow well in different environmental habitats and with its big, dynamic genome that provides great metabolic versatility (Klockgether et al., 2011). The various strains of *P. aeruginosa* pose high intrinsic resistance to antimicrobials and possess great ability to tolerate different biocides e.g. disinfectants, antiseptics and preservatives (Klockgether et al., 2011: Kampf, 2016: Botelho et al., 2019).

History

In 1882 Gessard isolated *Pseudomonas aeruginosa* from green pus for the first time (Palleroni, 2010). *Pseudomonas aeruginosa* belongs to the family Psedomonasdacease (Balasubramanian et al., 2013), and member of family Pseudomonaceae are present almost everywhere (ubiquitous) - in aquatic environment, plants, soil and few smaller mammals have been detected as their reservoirs (Gumey et al., 2020: Blanc et al., 2007: Klockgether and Tömmler, 2017: Spiers, 2000). *P. aeruginosa* can also be part of normal flora of skin and gastrointestinal tract. The Genus Pseudomonas was first described by German Botanist walther Migula in late 19th century. He observed the motile cells with spores resembling the nanoflagellate Monas spp. Therefore, the pseudomonas name was taken for the false identification "pseudo' meaning false (Migula, 1900). The word "aeruginosa" is taken for the characteristic color colonies on media defined by Schroeter in 1872, resembling green and name was given to the bacterium aeruginosum that was later placed in genus Pseudomonas by Migula (Palleroni, 2010). *P. aeruginosa* is an obligate aerobic, gram negative, rod-shaped bacterium, measuring 0.5 µm by 3.µm and motile by single polar flagellum (Ramos, 2011: Valles et al., 2004: Palmer et al., 2010). *P. aeruginosa* grows not only in normal conditions but also in oxygen deficient conditions and can colonizes wide range of environments (Kielhofner et al, 1992).

Biochemical Characters

Pseudomonas aeruginosa is not a fastidious (slow grower) bacteria that it can grow in normal growth environment. It can grow on non-selective media such as Blood agar, Luira-Betani, Nutrient agar and Mueller-Hinton agar but there are few selective media for selective isolation of *P. aeruginosa* cetrimide agar and King-A and B media. The surviving temperature range is wide from 4-40 ˚C (Brown and Lowbury 1965: Gajdács et al., 2019). The phenotypic features include the odor which is grape like or Fresh tortilla, hemolytic activity on blood agar, pigmented colonies on specific culture media help in fast identification of *P. aeruginosa* (Clark et al., 2015). It does not ferment glucose but produces Catalase and Oxidase enzymes (Nanvazdeh et al., 2013). The pigments such as pyoverdine (a green-yellow pigment also water soluble) secreted by 70 to 80% of isolates. This specific pigment pyoverdine turns as a siderophore when iron concentration is low. A blue-green pigment called Pyocyanin (lipid soluble) also plays important role in metabolism of iron and necessary for maintaining a nutrient equilibrium environment and in cell to cell communication. Another water-soluble pigment called pyorubin or red-brown pigment secreted only by 2-3% of *P. aeruginosa* isolates, play an important role in redox-equilibrium (Behzadi et al., 2008: Hall et al, 2016: Meyer et al., 1996: Alyydice-Francis and Brown, 2012). The pigment production in Pseudomonas spp. is induced by high concentration of phosphate (El-Fouly et al., 2015: Behzadi et al., 2011).

About the Genome of *Pseudomonas aeruginosa*

The genome of wild-type *Pseudomonas aeruginosa* strain was sequenced completely in 2000, which provided very important information regarding pathogenicity and resistance (Stover et al., 2000). In genome of *P. aeruginosa* there are 5570 Open reading frames which makes it the largest genome among prokaryotes. These high number of open reading frames are involved in encoding of unusually high amount of regulatory proteins, involved in transport and virulence, and make the *P. aeruginosa* strains very versatile and adaptive. Moreover, it was revealed that only 0.03% of genes are engaged in antimicrobial resistance and 10% of genes fall in the "pathogenicity islands" which consists of genes required for virulence determinants and also acquire mobile genetic parts that code for resistance (Kipnis et al., 2006: Woods, 2004: Normark, 2002).

The complete sequencing of *Pseudomonas aeruginosa* revealed that its genome consists of regulatory genes for efflux pumps, other transportation factors, locomotion, chemotaxis and certain genes which direct metabolic pathways that allow the bacterium to adjust in different conditions, and genes that code for an overabundance of virulence and antimicrobial resistance determinants (Brüggemann et al., 2018: Bao et al., 2014). One major example is the impairment in cystic fibrosis transmembrane conductance regulator genes (CFTR) that succinate is accumulated in the lungs and used as nutrient source by *Pseudomonas aeruginosa* for its colonization and growth in lungs (Jansen et al., 2016). Among secretory virulent factors proteases are as trademarks in Pseudomonas pathogenicity that make up approximately 3% of the ORFs in PAO1 genome (Vodovar et al., 2006: Stover et al., 2006). The horizontal gene

transfer of transposons has increased the diversity in *Pseudomonas aeruginosa* genome (Bonomo et al., 2006). There is an intrinsic method of increasing the genetic variability in mutable strains of Pseudomonas aeruginosa. For the repair of mismatching DNA bases microorganism has a trimer (MutS-MutL-UvrD timer), which maintains the integrity of genome in microorganisms (Hogardt et al., 2013: Rees et al., 2019). The rate of spontaneous mutation is increased to 1000, if there is defect in repair system that result in "hypermutator" strains. In isolates from cystic fibrosis it is principally observed that strains show unusual phenotypic changes "mucoid stage" and show high antimicrobial resistance (Ciofu et al., 2011: Lee et al., 2011).

Virulent determinants of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa can survive in harsh conditions by secreting different virulence factors, which also help in causing infections (Vidaillac & Chotirmall, 2021: Azuama et al., 2020: Shaw, & Wuest, 2020: Sadikot et al., 2005; Driscoll et al., 2007). The virulence factors produced by *P. aeruginosa* such as Fimbriae, Polar flagella, type three secretion system, some enzymes Elastase, protease, hemolysin and some quorum-sensing molecules (Sadikot et al., 2005; Driscoll et al., 2007). The outer and important virulent factor called lipopolysaccharide (LPS) acts as a guard to cells and its endotoxic part lipid A causes tissue damage, helps in attachment, and binding to host receptors (Park et al., 2022). LPS also plays role in antimicrobial resistance and biofilm formation (Chambers et al., 2017). The outer membrane proteins are important in the exchange of nutrients, attachment and antimicrobial resistance (Sabnis, et al., 2021). Additionally, the antimicrobial resistance due the biofilm formation is linked with flagella, pilus, and other attachment molecules. The secretory system of *Pseudomonas aeruginosa* are pili-associated (T4SS), flagella-associated (T6SS-linked) and type 3 secretory system (T3SS), which support attachment and colonization, motility and swarming and react to chemical signals. Furthermore, the main role in biofilm formation is of exopolysaccharides e.g. psl, pel and alginate (Ozer et al., 2021).

The *Pseudomonas aeruginosa* causing acute infections are very different phenotypically from those which cause chronic infections (Smith et al., 2006). The isolate that cause chronic infection lack certain virulence determinants such as pili and flagella or type three secretion system as compared to the isolates causing acute infections express many virulence determinants (Hogardt & Heesemann, 2010). The chronic infection causing, isolates are biofilm forming isolates and express great amount of exopolysaccharide that make them mucoid strains (Sadikot et al., 2005; Kipnis et al., 2006). The strains of *P. aeruginosa* can cause both invasive and toxigenic infections due to the presence of virulent factors on surface which allow attachment, colonization and invasion and also release certain molecules which cause damage to tissues or cause over production of cytokines (Kipnis et al., 2006).

Secretory structures as virulent determinants/Factors

The secretory system in *Pseudomonas aeruginosa* is very complex, which consists of certain virulent factors such as elastases, proteases, toxins and lipases that are secreted both inside and outside of the host cell (Ma et al., 2003). The *Pseudomonas aeruginosa* is responsible

for the cause of different hospital acquired infections and secretory virulent determinants play important role in communicating with the host (Rasko & Sperandio, 2010). These virulent factors enable the pathogen to degrade or escape from host immune cells and allow bacterial colonization and proliferation (Ribet & Cossart, 2015: Sharma et al., 2017: Korotkov et al., 2012). So far six different secretory systems has been reported in pseudomonas aeruginosa, which are categorized into two categories. The One-step secretory system consists of T1, T3, T4 and T6, while Two-step secretory system comprises of T2 and T5 secretory system. The mechanism of one-step secretion system is that the proteins are secreted directly on the surface from cytosol, while in the second type the proteins stay in the periplasmic space for a while and then carried out of the bacterium (Qin et al., 2022).

Category One

1. **T1SS:** This type is the simplest and extensively studied secretory system. There are three parts of T1SS: (i). Inner membrane protein-IM, (ii). ABC transporter and (iii). An outer membrane protein-OMF. These give energy to all process and are connected with periplasm with the help of an adopter protein (Delepelaire, 2004). The T1SS in *P. aeruginosa* is described in two different systems (i). Apr system and (ii). Has system (Filloux, 201: Delepelaire, 2004: Ma et al., 2003). The Apr system further consists of three components such as an ABC transporter (AprD), AprE (adaptor), and OMF (AprF) and are associated with alkaline protease AprA & AprX secreted outside of the cell (De Sousa et al., 2021), this is involved in numerous infections caused by *P. aeruginosa* (Matsumoto, 2004). The Has system is also comprised of (ABC transporter) HasD, (adaptor) HasE, and OMF (Alav, 2021). The Has proteins have the ability to sequester iron from blood by binding with hemoglobin when there is iron deficiency in the early stages of *Pseudomonas aeruginosa* infection (Cassat & Skaar, 2013: Delepelaire, 2004). Type 1 secretory system (T1SS) is also observed in many other bacteria such as Neisseria meningitidis, E. coli, *P. aeruginosa* and Salmonella enterica (Thomas et al., 2014). Hence, considering the data available to understand the mechanism of T1SS is inadequate, therefore requires further revelation to understand its role in pathogenesis of pseudomonas infection.
2. **T3SS:** This appears like a molecular needle with five different components which secrete the effector proteins outside of the cytosol (Coburn et al., 2007: Hauser, 2009), and it is also linked with the assembly of flagella (Bleves et al., 2010). The five components of T3SS are a needle composite, the transporter system, the controlling proteins, the working proteins and chaperones. This secretory system plays a vital role in virulence of *Pseudomonas aeruginosa* and was discovered for the first time in 1996 (Coburn et al., 2007). Thirty six genes are involved in the complexity of T3SS that are grouped into five operons on chromosome of pseudomonas aeruginosa. The T3SS is formed and regulated by these thirty six genes (Ma et al., 2003: Soscia et al., 2007: Hauser, 2009: Frank et al., 1997). These five operons are named as the exsD-pscB to pscL, pscN to pscU, pcrGVH-popBD, popN-pcr1-pcr2-pcr3-pcr4-pcrD-pcrR OPR, and last one is exsCEBA operon (Soscia et al., 2007: Hauser, 2009). Different type of virulence proteins are released in Type III secretory system such as (ExoS, ExoT, ExoY, and ExoU), which also

damage the cell to cell communication system and become cytotoxic at different stages (Francis et al., 2002: Zhu et al., 2016: Sawa et al., 2014).

The T3SS is controlled in two stages: firstly, decoding of T3SS genes and secondly, commencing of secretions. These two stages are turned on when bacteria is in touch with host cells that leads to increased production of T3SS proteins (Urbanowski et al., 2005, McCaw et al., 2002). The calcium concentration is a determining factor in activating the transcription of T3SS genes (Bleves et al., 2010). These genes are regulated by ExsA regulator master (Brutinel et al., 2008). The binding site of ExsA is an upstream nucleotide sequence for the polymerase enzymes on T3SS promotor (Ahator and Zhang, 2019). The expression of ExsA is self-regulated due to the binding with exsCEBA promotor (Vakulskas et al., 2009). ExsD gene is anti-activator that stops the transcription which is exsA-dependent (McCaw et al., 2002). There is an anti-anti-activator which binds with exsD when calcium concentration is low and stimulates ExsA inactivation, therefore, permitting binding with promotor region (Dasgupta et al., 2002). The exsE is an anti-anti-anti-activator which binds with exsC inhibiting from exsD (Rietsch et al., 2002). The intracellular section of exsE helps the complexes of ExsE-ExsC and ExsA-ExsD, to avoid ExsA related transcription (Dasgupta et al., 2002: Rietsch et al., 2002). The T3SS operon is also controlled by few other upstream regulators such as cAMP biosynthesis (Wolfgang et al., 2003), a universal controlar, Vfr, and ResT/LadS/GacAS (Ahator and Zhang, 2019: Yahr and Wolfgang, 2006). Besides these, in *Pseudomonas aeruginosa* T3SS genes are also regulated by many other factors. In PAO1 the MgtC and OprF control the T3SS and damage to macrophages induced by ExoS (Garai et al., 2019). There are two types of regulation mechanism; positive and negative regulation. The PsrA, (Shen et al., 2006), HigB (Li et al., 2016), Vfr (Marsden et al., 2016), and DeaD (Intile et al., 2015) regulate the positive regulation system. The negative regulation is controlled by AlgZR, GacAS (Goodman et al., 2004), MexT (Jin et al., 2011), and MgtE (Chakravarty et al., 2017).

Another important activator for T3SS gene expression in *Pseudomonas aeruginosa* is spermidine transporter which allows the bacterium to detect and sense the molecules involved in communicating and signaling in host tissues (Zhou et al., 2007). If the genes of spermidine transporter are deleted, the gene expression of T3SS which are dependent on host-cell will be inhibited (Ahator and Zhang, 2019). The spermidine is grouped in polyamines which are involved in stress response, tolerating acids and antimicrobial resistance (Iyer et al., 2003: Kwon and Lu, 2006: El-Halfawy and Valvano, 2015). According to multiple researchs which show that either antimicrobial resistance is induced or increased by polyamines such as resistance of *Pseudomonas aeruginosa* against aminoglycosides and quinolone. On the other side, it is also revealed that spermidine received from outside and some polyamines can increase susceptibility to certain antibiotics such as lactam antibiotics, chloramphenicol, nalidixic acid and trimethoprim with the help of efflux pump and LPD operon (Kwon and Lu, 2006). If spermidine signals are identified it could give a method for the control of pseudomonas infection.

3. **T4SS:** T4SS consists of multiple subunits anchored in cell membrane that stands responsible for transferring different protein complexes out of the cell. It is ancestrally linked with horizontal

gene transfer-induced antimicrobial resistance and virulence (Juhas et al., 2008; Lawley *et al*., 2003; Christie *et al*., 2005). The genes that encode for T4SS are grouped into a single working unit. These genes are significantly different in many ways such as gene sequence, gene content and homology. Based on these similarities and evolutionary relationships the T4SS can be categorized into two different schemes (Lawley *et al*., 2003; Christie *et al*., 2005). Initially, the T4SS classification was divided into three types as F, P and I; this classification was on the basis of mismatching with conjugative plasmid representative (Lawley *et al*., 2003). In another classification the F and P type are categorized together in IVA-type secretory system. The IVA-type secretory system of *Pseudomonas aeruginosa* is also present and resembles with the VirB/VirD4 of Agrobacterium tumefaciens (Juhas et al., 2008; Christie *et al*., 2005). The secretory system components which were different from F & P types are placed in IVB types and its genetic factors resemble with the Dot/Icm system of Legionella pneumophila. There is third secretory type that has no resembles with neither IVA nor IVB system (Christie *et al*., 2005).

The Type-4 secretory system consist of pilin units-major and minor and PilA. The prepilin peptidase enzyme processes both major and minor pilin units. T4SS and T2SS possess an evolutionary association with each other which impacts the assembly of pili. The pili assembly, movement, and adhesion can be affected by minor pilins (Tammam et al., 2013). Ultimately, the main role of T4SS is related with potential pathogenesis of microbes through horizontal gene transfer (HGT).

4. **T6SS:** In *Pseudomonas aeruginosa* T6SS is a unique and vital virulence machinery, involved in antagonistic relationship with the surrounding microbes (Chen et al., 2020). In the beginning, the Hcp Secretion Island (HSI) encoding for T6SS were grouped into three gene groups but later on these were renamed as H1-T6SS to H3-T6SS types (Vettiger et al., 2016; Chen et al., 2015), which contain approximately 15-20 genes for each type (Bleves et al., 2010; Bingle et al., 2008). The T6SS machinery consists of 13 essential components, separated into baseplate-resembling structure (Zoued, et al., 2014). Moreover, the T3SS structure also resembles with needle-like structure of T3SS (Allsopp et al., 2017). The appearances of T6SS structure is like an inverted bacteriophage tail, associated with Hcp (hemolysin coregulated protein) and Vgr (Valine-glycine repeat) composite, which act as a cell-piercing devices and help in extracellular release of molecules (Pukatzki et al., 2006: Hood et al., 2010). The Hcp 1 protein in *Pseudomonas aeruginosa* is crystal, hexameric and shares homology with the tube of T4 bacteriophage tail (gp19 protein). On the other side the N-terminals of Vgrs contains similarities with gp5 and gp27 proteins of T4 phage. These two proteins (gp5 and gp27) function for phage as a puncturing unit to enter its DNA in the cell (Bleves et al., 2010). Therefore, it can be said that the target site recognition and delivery of DNA is similar as the entry of phage genetic material in target cell (Kanamaru et al., 2010). T6SS also has few other components: a FHA working protein, ClpV ATPase, DotU, IcmF protiens and outer membrane lipoprotein (Bleves et al., 2010). In addition, *Pseudomonas aeruginosa* also gets energy from ClpV and an AAA+ family ATPase for the secretory mechanism (Basler, 2015).

The complete genome sequencing of *P. aeruginosa* has revealed that its genome contains three loci for the components (HSI-I, HSI-II and HSI-III) of T6SS (Bingle et al., 2008). T6SS also secretes six different effector proteins (Tse1-6). Two of them Tse 1 and Tse 3 are involved in breakage of peptidoglycan when released intracellularly, which ultimately results in bacterial cell lysis. The Tse2 functions to stop the growth of both bacteria and host. The remaining effector proteins Tse4, Tse5 and Tse6 play an important antibacterial role (Whitney et al., 2014; Russell et al., 2011). The HSI-1 type has a sharp edge structure similar to tail of bacteriophage, controls regulation of two-component and sRNAs (Russell et al., 2011; Basler et al., 2012). The HSI-II plays important role in endocytosis process by epithelial cells and HSI-III is key in pathogenesis (Lesic et al., 2009). The regulators of quorum sensing and transcriptional factor MyfR are important in regulation and expression of HSI-II and HSI-III (Allsopp et al., 2017). Furthermore, HSI-I and HSI-II in *Pseudomonas aeruginosa* cause infection both in plants and animals (Lesic et al., 2009).

The secretion of protein molecules through T6SS requires few other components such as cytoplasmic adaptor Tla3 is required for H2-T6SS complex to secrete Tle3 toxins (Berni et al., 2019). The T6SS machinery ((H1-T6SS and H3-T6SS) is regulated by GacAS/Rsm that activates RsmY/Z to halt the attachment of RsmA/RsmN with fha1/tssA1 (Huang et al., 2019). The TseF secreted by H3-T6SS helps in entry of PQS-Fe3+ complex into cell by fitting it with OMVs of Pseudomonas quinolone signal (PQS) (Li et al., 2022). Remarkably, it was observed that quorum sensing and expression of secretion system are co-connected. In *Pseudomonas aeruginosa* three loci of genes coding for (HSI-I, HSI-II, and HSI-III) by MvfR and LasR are controlled by Quorum sensing (Lesic et al., 2009). In PAO1 strains the QS regulators control the expression of H2-T6SS involved in Las and Rhl (Sana et al., 2012).

Category two

T2SS: This specific secretory type is an adoptable system. It is widely present in gram negative bacteria and its main role is to help in transport of proteins which are folded in periplasm (Costa et al., 2015: Naskar et al., 2021). It comprises of two phases to excrete extra cellular proteins: the phase one includes the Sec (Secretory) or Tat twin-arginine translocation (Cianciotto, 2005: Lee et al., 2006), to carry proteins from cytoplasm into periplasm, and second phase includes T2SS complex-mediated to delivery more proteins into extra cellular spaces (Cianciotto, 2005). The extracellular protein are first delivered to inner membrane where stored for short time in periplasmic space and then transported outside (Nivaskumar and Francetic 2014: Green and Mecsas, 2016). The secretory (Sec) pathway comprises of different components; protein targeting & membrane incorporated channels (SecYEG translocase), a motor protein and proteins attached with SecB-specific singles sequence are transported outside of membrane (Veenendaal et al., 2004). In gram negative bacteria the Tat (twin-arginine translocation) system comprises of TatA and TatB which make the selection about retaining or transporting the protein outside with specific twin arginine motif (Goosens et al., 2014).

The type 2 secretory system consists of two types; first Extracellular protein (Xcp) system which secretes almost 14 different proteins e.g. proteases , lipases, and second type is called

Homologous to Xcp system, this type is active only in phosphate deficient conditions (Ball et al., 2002). The second type is only involved in secretion of alkaline phosphatase. In coding of Xcp at leaset 11 genes are involved that are ordered into two different operons from xcpP to Q and xcpR to Z (Bleves etal., 2010: Rybtke, et al., 2015). Some enzymes such as elastase (LasB, LasA), lipases (PlcN, PlcH, LipA and Lipc), phospholipases and exotoxin A (ToxA), these all play important role during infection. The elastases cause degradation of connective tissues especially in lungs (Casilaget al., 2016). The pulmonary system is also affected, lipases and phospholipases breakdown the fats present in membranes of these organs which can change the role of these organs in immunity (Korotkov et al., 2012: Ostroff et al., 1990). In *Pseudomonas aeruginosa* Exotoxin A is another significant virulent factor that it stops the ADP-ribosylation in protein synthesis, which results in cell death (Allured et al., 1986). It is further revealed through a study on hospital wastewater for the identification of genes (popB, lasB, lasA, and tox A) involved in virulence of Pseudomonas aeruginosa. It shows a great public health threat that these virulent factors may stimulate adaptability of microorganisms in environment which may increase their pathogenicity (Mapipa et al., 2021).

T5SS. This system is one of the simplest secretory pathway defined, comprised of five autotranspoters (type Va to Ve), which transport proteins outside of membrane (Costa et al., 2015: Bleves et al., 2010). In this system through proteolytic breakdown proteins are degraded and then transported outside (Filloux et al., 2011). This system is comprised of single polypeptide chain, attached with a translocator β-barrel and an exit way for extra cellular secretion (Meuskens et al., 2019: Bleves et al., 2010). Through T5SS different protiens EstA, LepB, and LepA are secreted. Among these proteins EstA has possess a transporter esterase activity, present on outside of membrane and plays role in rhamnolipid that can influence motility of cell and biofilm formation (Wilhelm et al., 2007: Meuskens et al., 2019).

Locomotion as virulence factor (Motility)

The attachment of microorganisms on abiotic surfaces e.g. medical instruments, and on biotic surfaces e.g. wounds and tissues, is very important for causing infections. Motility is important in formation of biofilms which lead to problematic and complex microbial communities, showing great resistance against many antimicrobials (O'May et al., 2011). Flagella, fimbria and pili are used for locomotion by Pseudomonas aeruginosa. Type IV pili is used for attachment and motility on solid surfaces called twitching motility, while flagellum is used for motility in broth media (Harshey, et al, 2003).

Pseudomonas aeruginosa moves with the help of single polar flagella, made up of protein flagellin also called FLiC. (Wu et al., 2015: Miao et al., 2007). Several short appendages called pili (type IV) are also used for motility. The flagella and pili both can be used as adhesins and as a motility organ. These can also start an inflammatory response. The flagellar structure is comprised of three parts, a basal body, a rod originating from cell wall and the hook. The swimming and swarming movements of *Pseudomonas aeruginosa* due to rotation of flagellum. When flagellum rotates anti-clockwise the bacterium moves forwards and clock wise motion

causes tumbles. The chemical signals trigger the directions of movements (Guttenplan, and Kearns, 2013: Miao et al., 2007: Harshey, et al, 2003).

Quorum sensing and biofilm formation

Quorum sensing is termed as social interaction of bacteria, during its development autoinducers are released which are tiny molecules that effect the gene expression in cell to cell communication (Miller & Bassler 2001: Pesci & Iglewski, 1997: Mukherjee & Bassler: 2019). When the bacterial population increases to a certain threshold, certain behavioral changes occur in the bacteria which helps them to fight for the available ecological niche (Turanet al., 2017: Rampion et al., 2014). In *Pseudomonas aeruginosa* the biofilm development requires great coordination within the densely packed bacterial communities (Yan & Wu, 2019).

Biofilms are produced when bacteria attach to surface in a highly organized and structured microbial communities and are linked with Quorum sensing (Bjarnsholt et al., 2010: Yang et al., 2011). The extracellular polymeric substances (EPS) such as polysaccharides, lipids, nucleic acids and proteins make a sheath around these microbial communities. The majority of biofilm around 50-90% are made up of these extracellular polymeric substances (EPS). These EPS also provide robust resistance to chemical and mechanical forces such as flowing water and make the toxic chemicals & antibiotics impermeable (Hall-Stoodley & Stoodley, 2009; Lieleg et al., 2011; Høibyet al., 2011).

Quorum sensing circuits of *Pseudomonas aeruginosa*

In *Pseudomonas aeruginosa* there are four quorum sensing circuits which are Las, Rhl, PQS and IQS and there auto inducers are N-3-oxo-dodecanoyl-L-homoserine lactone (3O-C12-HSL), N-butyryl-L-homoserine lactone (C4-HSL), 2-heptyl-3-hydroxy-4-quinolone (PQS) and 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS), respectively, produced intracellularly. The QS circuits are structured in hierarchy such as Las at the top of the sequence (Lee & Zhang, 2015). The first two QS circuits Las and Rhl are activated in the start of log phase with augmented cell density, whereas the other PQS and IQS circuits are triggered in the end of log phase (Choi et al., 2011) mainly when there is iron depletion (Oglesby et la., 2011) and phosphate depletion conditions (Rampioni et al., 2016), respectively. The two well studied QS circuits in *Pseudomonas aeruginosa* are Las and Rhl, the Las circuit contains specific transcriptional activator and AI enzyme LasR & LasI, respectively, which leads to synthesis of N-(3-oxydodecanoyl) homoserine lactone (3O-C_{12}-HSL), the signal molecule. The other QS circuit Rhl consist of transcriptional activator and AI synthase enzyme RhlR and RhlI, respectively, which leads to synthesis of N-butyryl homoserine lactose (C_4-HSL). It is found in different studies that biofilm development in *Pseudomonas aeruginosa* is poorly observed in las signal molecule deficient strains (Davis et al., 1998). The AIs go through membrane tracking which is focused outside of the cell then inside, seemingly initiated by diffusion, efflux pumps or through outer membrane vesicles (Mashburn, & Whiteley 2011). The transferred 3O-C12-HSL is then attached with LasR the regulator protein and this complex triggers LasI synthase gene (Seed et al., 1995). The expression genes involved in RhlR, RhII, pqsR and pqsABCDH which code RHL are induced by LasR–3O-C12-HSL (Lee & Zhang, 2015). The pqsABCDH genes in are

activated by PqsR-PQS composite and also start RhlRI gene expression (McKnigh et al., 2000). The RhlR which is believed to balance the ratio of 3-oxo-C12-HSL and C4-HSL can also inhibit both pqsR and ABCDH genes, therefore, it controls the initiation of PQS pathway (Cao et al.,, 2001).

In *Pseudomonas aeruginosa* the three important QS circuits such as Rhl. Las, and PQS produce certain functional elements which play a vital role in biofilm development. The functional elements are rhamnolipid (Pamp et al., 2007: Pearson et al., 1997), pyocyanin (Das et al., 2013: Das et al., 2015), pyoverdine (Banin et al., 2005), pel polysaccharide (Sakuragi & Kolter, 2007: Da Silva et al., 2019) and lectins (Da Silva et al., 2019: Diggle et al., 2006). Rhamnolipids contain glycolipidic compound which help to preserve the pores and channels in microbial colonies which help in passing the liquid and food inside the developed biofilms. The iron is sequestered by Pyovedine and delivered for the development of biofilms. The iron depletion favors twitching motility as compared to sessil growth which hinders biofilm formation (Visca et al., 2007). Pyocyanin is not required for the growth of pseudomonas aeruginosa, rather it is released as secondary metabolite with cyctotoxicity that it helps in lysis of cell and releasing eDNA which is an important component of biofilm development. It can bind with eDNA to increase its viscosity and promotes cell aggregation in biofilm development in the environment. Through cationic reaction eDNA is attached with pel polysaccharide which enhances the interaction in biofilm matrix. Lactins occurs in two forms as soluble proteins i.e. LecA and LecB. Lectins contain adhesive characteristic which help in holding both cells and exopolysaccharides in developing biofilm. In all, these interaction at molecular and cellular level lead to formation of full-bodied and settled biofilm.

Formation of Biofilm

The bacterial growth and biofilm formation depends on two important factors the availability of nutrients and water (Schwartz et al., 2009). The social interaction and supportive behavior of microbes with each other helps them in formation of complex biofilm development (Lee, et al., 2014). Mostly biofilms are formed in result of polymicrobial interactions, therefore, among the microbes a big competition occurs for available nutrients and space. The polymicrobial growth on solid surfaces encourages cooperation in the form of horizontal gene transfer, metabolic help, and other interactions. Hence, it proves to be difficult to treat and which increases antimicrobial resistance (Wolcott et al., 2013: Yang et al., 2013).

Composition of Biofilm

The three exopolysaccharide which are highly involved in formation and architect of biofilm are Psl, Pel and alginate (Ghafoor et al., 2011: Billings et al., 2013). The Psl is a neutral polysaccharide composed of d-glucoase, d-manose, and I-rhamanose units (Ma et al., 2009: Byrd et al., 2009). This particular polysaccharide is important for the attachment on surfaces, cell to cell communication in both mucoid and non-mucoid strains for the formation of biofilms (Ghafoor et al., 2011: Ma et al., 2012: Jones et al., 2017). Following features are observed in psl (i). It is beneficial for biofilm forming strains as compared to free floating strains, (ii). Non-Psl generating stains can grow better in mixture with psl producing cells, (Billings et al., 2013: Irie

et al., 2017). (iii). in the process of biofim formation the Psl negative cells are dominated by Psl positive cells; and (iv) the Psl producers are not exploited by non-producers (Irie et al., 2017). When the biofilm formation is matured the psl appears in mushroom-like shape and provides structural stability (Ma et al., 2009). High psl production is connected with aggregating cells in liquid medium and this characteristic is also observed in sputum of CF patients (Staudinger et al., 2014: Irie, et al., 2012). Psl is also a signaling molecule that promotes c-di-GMP (bis-(30-50)-cyclic dimeric guanosine monophosphate) production whose raised concentration helps in thicker and fully bodied biofilms (Irie, et al., 2012). Additionally, psl protects the cells from antimicrobials (Billings et al., 2013), and from phagocytic activity of neutrophils (Mishra et al., 2012) which helps in insistent infection.

The other component of biofilm is Pel which is a cationic polymer of partly de-acelyted N-acetyl-d-glucosamine and N-acetyl-d-galactosamine. Pel is also an important component in non-mucoid strains which helps in surface attachment and helps in maintaining biofilm integrity (Colvin et al., 2013: Jennings et al., 2015). Pellicle biofilm is formed due to Pel which is made on the surface of a stationary broth culture (Friedman, and Kolter et al., 2004). The surrounding condition play important role in formation of Psl and Pel as both are strain-specific (Colvin et al., 2011). Pel increases the tolerance in biofilm embedded cell from aminoglycoside antibiotics (Yand et al., 2011). Moreover, the biofilms containing Pel are stubborn to Colistin and phagocytic activity of neutrophils (Baker et al., 2015).

The third major component of biofilm produced from mucoid strains of Pseudomonas because of the mutation in mucA22 allele. The mucoid characteristics are frequently found in CF isolates that demonstrate of changing to chronic from acute infection (Ciofuet al el., 2015: Folkesson et el., 2012). Alginate is composed of anion acetylated polymer of mannuronic acid and guluronic acids (Evans et al., 1973). The alginate has many important functions such as helps maturation of biofilm, protects from phagocytes and opsonizing molecules, and makes biofilm surfaces impermeable to antibiotics (Strempel et al., 2013: Tseng et al., 2013; Hay et el., 2013; Hay et al., 2010). The ratio of mannuronic acid and guluronic is important in biofilms because they affects their viscoelastic properties which become obstacle in treating cough in Cystic fibrosis caused by *Pseudomonas aeruginosa* (Gloag et al., 2018; Wloka et al., 2005; Reh, and Valla1997).

Development of Biofilm

The biofilm formation takes few hours to form (Dufour et al., 2010). The biofilm formation is completed in 5 steps: (I). Reversible attachment of free-floating bacteria with solid surfaces, (II). Irreversible attachment of cells with solid surface and aggregation of cells, (III). Initial formation of biofilm (IV). Formation of micro colonies to form mature biofilm, (V). Dispersion of cells from micro colonies to further spread the infection (Jamal et al., 2018: Lewis, 2001).

Stability of biofilm

The stability and firmness of *Pseudomonas aeruginosa* biofilms depend on different polysaccharide secretions such as alginate, psl, and pel (Ghafoor et al., 2011: Ryder, et al., 2007). The major polysaccharide component alginate is composed of D-mannuronic acid and L-

glucuronic acid in the form of unbranched polymer. The stability and protection of biofilm depends on this vital polymer. It is also involved in the preservation of water and nutrients (Rasamiravaka et al., 2015). The pel matrix is full of glucose and psl polysaccharide consists of repeating units of D-mannose, L-rhamanose and D-glucose. Both pel and psl are involved in initial stages of biofilm development (Ma et al., 2012: Colvin et al., 2012: Franklin et al., 2012). In *Pseudomonas aeruginosa* eDNA is another important part of biofilm which serves as a major source of nutrient for the bacteria in the biofilm matrix and play necessary role in cell to cell communication (Flemming & Wingender, 2010: Mulcahy et al., 2010.

Other virulent determinants

Proteases

The *Pseudomonas aeruginosa* secretes several enzymes which are known as proteases that play role in eye infection and in sepsis, in which these proteases breakdown antibodies & fibrin and also disturb epithelia tight intersections (Kipnis et al., 2006). Whereas their role in lung infection is unclear, but these proteases can damage respiratory tissues such as breaking down the lung surfactant in host (Fleiszig & Evans, 2002; Hobden, 2002; Kipnis et al., 2006).

Alkaline protease a zinc metalloprotease causes degradation of complement proteins and fibronectin in host (Laarman et al., 2012). This particular protease helps *Pseudomonas aeruginosa* to escape from immune cells by interfering with flagellin signaling by breaking down flagellin single units (Bardoel et al., 2011).

There are two elastases in *Pseudomonas aeruginosa* LasA and LasB, controlled by lasI quorum-sensing and excreted by type-2 secretory system (Toder et al., 1994; de Kievit & Iglewski, 2000). The LasB is used as synonym for ‘elastase’ and LasA for ‘staphylolysin. The LasA is a serine protease and can breakdown the peptidoglycan stabilizing bridge made of penta-glycine in cell wall of staphylococci, with little proteolytic activity and can help LasB in protein breaking activity (Toder et al., 1994; Matsumoto, 2004). LasB can also breakdown the opsonizing surfactant lung proteins A & D (Mariencheck et al., 2003). In the end it is concluded that DlasB mutants are less resistant to phagocytosis (Kuang et al., 2011).

Lipopolysaccharide

Lipopolysaccharide is present as outer covering as a glycolipid which possess a great role in inducing immune response, inflammatory response, removing external molecules and starts contact with antibiotics (King et al., 2009). In gram negative bacteria such as *Pseudomonas aeruginosa* the lipopolysaccharide is composed of Lipid A molecules, core chain of polysaccharide and O-side chain (O-antigen). The major role of lipopolysaccharide is in antibiotic resistance. In *Pseudomonas aeruginosa* two components of lipopolysaccharide Lipid A and O-polysaccharide play important role in developing infections (King et al., 2009: Lam et al., 2011). The lipid A side chain can attach with CD14 and MD2 of host receptors which activates the production of inflammatory inducing cytokines and chemokines their overproduction can

lead to endotoxic shock (Teghanemt et al., 2005; Akira et al., 2006). Furthermore, any modification in lipid A can change bacterium's susceptibility to Polymyxin, antimicrobial peptides with positive charges and its inflammation causing characteristics. Penta and hexa-acylated lipid A forms are exhibited in laboratory grown *Pseudomonas aeruginosa* which are different due to decanoic acid at the position-three. In laboratory strains pentaacylated species are leading (c. 75%) in isolates from short and sudden infections (King et al., 2009). On contrary isolates from chronically CF patients exhibit hexa and few time hepta-acylated species with augmented inflammatory properties (Ernst et al., 2007). The change in binding of lipid A to MD2 receptor causes augmented inflammatory potency due to increasedacylated lipid A (Teghanemt et al., 2005) and adding of aminoarabinose to phoQ mutant strains can also results in increased inflammatory lipopolysaccharide (Gellatly et al., 2012). Certain changes as discussed above and especially aminoarabinose addition results in increased resistance to positive charged antimicrobial peptides such as Polymyxin (Ernst et al., 1999). In *Pseudomonas aeruginosa* O-antigen (O-polysaccharide) can occur simultaneously in two discrete form, structurally and serologically. The common A-band is made up of similar polysaccharide units of D-rhamnose which are extended nearly 70 sugar long and trigger very weak immunoglobulin response. In compare to, the B-band (O-specific) chain of polysaccharide is made of different units and it can trigger very strong immunoglobulin response (King et al., 2009). Some strains of *Pseudomonas aeruginosa* are called 'rough strains' as they do not produce O-polysaccharide and some are 'semi rough' as they replace the lipid A with only single O-saccharide unit (Hancock et al., 1983).

Antimicrobial resistance

The *Pseudomonas aeruginosa* infections are caused due to multiple intrinsic virulent determinants e.g. LPS, pili, alginate, etc. and few secretory virulent determinants such as pyocynic, proteases, siderophores, exotoxins etc. (Strateva, et al., 2011). Among the different resistance mechanisms in *Pseudomonas aeruginosa* such as enzymatic degradation & hydrolyzation of antibiotics (Vatcheva-Dobrevska et al., 2013; Fisher and Mobashery, 2014; Hakemi Vala et al., 2014), low absorption through membrane (Eren et al., 2013; Zgurskaya et al., 2015), out pouring of antimicrobials through efflux pumps (Poole, 2004; Aghazadeh et al., 2014), and the biofilm formation remain important mechanisms (Mah et al., 2003: Hancock e t al., 1998). Moreover, the great diversity in antimicrobial resistance is due to its versatile and big genome, and living in aquatic environment also contributes in acquiring resistance genes (Vaisvila et al., 2001). The complex extra cellular matrix decreases the effectiveness of antibiotics and detergents (Mah et al., 2003). Hence, *Pseudomonas aeruginosa* remains a major problem due to its antimicrobial resistance and biofilm formation (Buhl et al., 2015; Oliver et al., 2015). The resistant strains causing infections are still a major threat in the world as these infections cause 3-fold high mortality, 9-fold secondary bacteremia and 2-fold increase the stay at hospital which costs high economic burden (Giamarellou, 2002).

Major Mechanism of resistance in *Pseudomonas aeruginosa*

The most important and major resistance mechanism are three;

Impermeability of membrane

The antibiotics prescribed for the treatment of pseudomonas infection in order to reach their target site they have to cross the cell wall. The intrinsic concept of resistance in *P. aeruginosa* is attributed with impermeability of cell wall. The antibiotics fail to reach their target, never accumulate with host cell and those are also removed by efflux pump reaction.

The Alginate as a blockade

As discussed earlier In *Pseudomonas aeruginosa* the production of Alginate bring firmness and stability in biofilm structure. Alginate binds with cationic antimicrobials e.g. aminoglycosides and prevents its diffusion (Nichols et al., 1998) but this does not apply on mucoid strains as some of those strains are completely sensitive to aminoglycosides (Ciofu et al., 2001).

The outer membrane as a blockade

In *Pseudomonas aeruginosa* the outer membrane plays an important role in restricting the diffusion of antibiotics. It also act as a barrier to decrease the penetration rate of small hydrophilic molecules and reject larger ones. These specific small molecules like beta-lactums and DNA inhibitors (quinolones) only diffuse though the aqueous passages of porin proteins. These barrel-shaped molecules act as a trimer which span through the outer membrane. There are many porins in *Pseudomonas aeruginosa* and oprF is the major one which is present in all strains (Brinkman et al., 2000). The absence of oprF has not been found responsible for antibiotic resistance because such mutant strains possess limited ability to diffuse hydrophilic molecules. On the other hand OprD is a specific porin and plays a vital role in diffusion of amino acids containing positive charge and without oprD *Pseudomonas aeruginosa* become resistant to imipenem. Lacking oprD results in minimum inhibitory concentration from from 1–2 to 8–32 mg/L and also increases rate of resistance up to 17% (Livermore, 2001). Interestingly, the loss of oprD does not affect the resistance to meropenem which means that may cross via different pathway. The porin channels does not help aminoglycosides and Colistin to cross the outer membrane. As an alternative their uptake is promoted by attaching to the lipopolysaccharide and this attachment finishes the permeability barrier and permits the antibiotics to enter via the wall of plasma membrane. As the target site of aminoglycosides is at ribosomes so they are actively transported into the cells and Colistin exhibit their bactericidal activity via disrupting the plasma membrane. Due to an overexpression of outer membrane protein in laboratory strains of *Pseudomonas aeruginosa* exhibit resistance to aminoglycosides and Colistin. But such resistance have not been widely reported in clinical isolates of *Pseudomonas aeruginosa* (Gilleland et al., 1989).

The efflux system as resistance mechanism

This particular resistance system consists of three protein constituents; (i) a pump present in the cytoplasmic membrane and requires energy, (ii). A porin protein on the outer membrane and (iii), a linker protein which combines the other two components (Nikaido, 1998). This triple organization of protein constituents form an efficient extrusion system which pump out the toxic

molecules out of the cell. (Poole, 2001). There are twelve genes which code for efflux pumps identified in PAO1, each of it display specificity to substrate (Stover et al., 2000). However, among other efflux systems only five of them play role in resistance in clinical strains, MexAMFPMexBRND-OprMOMF,MexXMFP-MexYRND-OprMOMF,MexCMFP-MexDRND-OprJOMF, MexEMFP-MexFRND-OprNOMF, and MexJMFP-MexKRND-OprMOMF (Lister et al., 2009; Li et al., 2015). All the antibiotics are extruded by one or more then of these efflux system except polymyxins. The MexAB-oprM exiles the Beta-lactams, DNA inhibitors and a wide range of disinfectants. The aminoglycosides are extruded by MexXY-oprM, carbapenems and quinolones are extruded by mexEF-orpN. The genes for the expression of efflux system are present in all strains but expressed only in certain strains. However, the overexpression could be due to mutation in regulatory genes e.g. mexR that manages expression of mexAB-oprM genes (Ziha-Zarifi et al., 1999).

Inactivation and structural changes in antibiotics

AmpC gene is present in all Pseudomonas aeruginosa strains for chromosomal encoded beta-lactamases. But only induction is not responsible for resistance in CF strains. The enzymes over expressed can result in spontaneous mutation in ampR a regulatory gene. This phenomenon has been observed when there is high reliance on ceftazidime therapy (Giwercman et al., 1990). This enzyme is present in the periplasm and can been detected in sputum in antipseudomonal therapy (Giwercman et al., 1992). The ampC beta-lactamases pose great threat to cephalosporins when its production is increased. The other beta-lactamases including ESBLs produced by Pseudomonas aeruginosa are active against penicillin and cephalosporins (Korten et al., 2001). The clavulanic acid with ticarillin and tazobactaam with pipracillin as a beta-lactam inhibitor can give protection to some of the enzymes coded by plasmid but not from ampC enzyme (Maiti et al., 1998).

The aminoglycoside inactivation occurs by the transfer of acetyl, phosphate or adenylyl group by enzyme production to hydroxyl and amino group in antibiotics. The inactivation was considered as the main machinery of resistance against antibiotics before the discovery of that aminoglycosides are also susceptible to efflux pumps. The genes required for modification of enzymes are required to be transferred from strains possessing plasmids (MacLeod et al., 2000).

Conclusion

The antimicrobial resistance (AMR) remains a major public health issue. The main drivers of antimicrobial resistance are use, misuse and overuse of antimicrobials. The antimicrobial resistance develops due to strong adaptive characteristics of microorganisms which leads to problematic scenario in treatment. Along with other major life-threatening bacterial pathogens *Pseudomonas aeruginosa* possess great intrinsic and adaptive characteristics to become multi-drug resistant to commonly prescribed antibiotics. There are different virulent determinants and resistance mechanisms in *Pseudomonas aeruginosa* which increase its importance and makes it a top priority for researchers. The emergence of multidrug resistance in strains of *Pseudomonas*

aeruginosa have reduced the effectiveness of commonly prescribed antibiotics and left the situation with minimum treatment options. The infections due to resistant strains are linked with high morbidity, mortality and treatment costs.

To combat the emerging challenges of antimicrobial resistance an inclusive approach is required, to involve all stakeholders (policymakers, researchers, healthcare professionals, clinicians and scientists), to make sure robust surveillance system, infection control measures, antimicrobial stewardship and to spread public awareness about the importance and consequences of antimicrobial resistance (AMR). Additionally, the continuous research and development in field of finding new and alternative therapeutic agents e.g. antimicrobial peptides, bacteriophages, efflux pump inhibitors, can also play a major role to remain ahead of *Pseudomonas aeruginosa*'s evolutionary strategies.

References

1. Aghazadeh, M., Hojabri, Z., Mahdian, R., Nahaei, M. R., Rahmati, M., Hojabri, T., & Pajand, O. (2014). Role of efflux pumps: MexAB-OprM and MexXY (-OprA), AmpC cephalosporinase and OprD porin in non-metallo-β-lactamase producing Pseudomonas aeruginosa isolated from cystic fibrosis and burn patients. *Infection, Genetics and Evolution*, *24*, 187-192..
2. Ahator, S. D., & Zhang, L. (2019). Small is mighty—chemical communication systems in Pseudomonas aeruginosa. *Annual review of microbiology*, *73*, 559-578.
3. Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell*, *124*(4), 783-801..
4. Alav, I., Kobylka, J., Kuth, M. S., Pos, K. M., Picard, M., Blair, J. M., & Bavro, V. N. (2021). Structure, assembly, and function of tripartite efflux and type 1 secretion systems in gram-negative bacteria. *Chemical Reviews*, *121*(9), 5479-5596.
5. Allsopp, L. P., Wood, T. E., Howard, S. A., Maggiorelli, F., Nolan, L. M., Wettstadt, S., & Filloux, A. (2017). RsmA and AmrZ orchestrate the assembly of all three type VI secretion systems in Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences*, *114*(29), 7707-7712.
6. Allured, V. S., Collier, R. J., Carroll, S. F., & McKay, D. B. (1986). Structure of exotoxin A of Pseudomonas aeruginosa at 3.0-Angstrom resolution. *Proceedings of the National Academy of Sciences*, *83*(5), 1320-1324.
7. Allydice-Francis, K., & Brown, P. D. (2012). Diversity of antimicrobial resistance and virulence determinants in Pseudomonas aeruginosa associated with fresh vegetables. *International journal of microbiology*, *2012*.
8. Azuama, O. C., Ortiz, S., Quirós-Guerrero, L., Bouffartigues, E., Tortuel, D., Maillot, O., ... & Tahrioui, A. (2020). Tackling Pseudomonas aeruginosa virulence by mulinane-like diterpenoids from Azorella atacamensis. *Biomolecules*, *10*(12), 1626.

9. Baker, P., Hill, P. J., Snarr, B. D., Alnabelseya, N., Pestrak, M. J., Lee, M. J., ... & Howell, P. L. (2016). Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent Pseudomonas aeruginosa biofilms. *Science advances*, *2*(5), e1501632..
10. Balasubramanian, D., Schneper, L., Kumari, H., & Mathee, K. (2013). A dynamic and intricate regulatory network determines Pseudomonas aeruginosa virulence. *Nucleic acids research*, *41*(1), 1-20.
11. Ball, G., Durand, É., Lazdunski, A., & Filloux, A. (2002). A novel type II secretion system in Pseudomonas aeruginosa. *Molecular microbiology*, *43*(2), 475-485.
12. Banin, E., Vasil, M. L., & Greenberg, E. P. (2005). Iron and Pseudomonas aeruginosa biofilm formation. *Proceedings of the National Academy of Sciences*, *102*(31), 11076-11081.
13. Bao, Z., Stodghill, P. V., Myers, C. R., Lam, H., Wei, H. L., Chakravarthy, S., ... & Swingle, B. (2014). Genomic plasticity enables phenotypic variation of Pseudomonas syringae pv. tomato DC3000. *PloS one*, *9*(2), e86628.
14. Bardoel, B. W., van der Ent, S., Pel, M. J., Tommassen, J., Pieterse, C. M., van Kessel, K. P., & van Strijp, J. A. (2011). Pseudomonas evades immune recognition of flagellin in both mammals and plants. *PLoS pathogens*, *7*(8), e1002206.
15. Basler, M. (2015). Type VI secretion system: secretion by a contractile nanomachine. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1679), 20150021.
16. Basler, Á., Pilhofer, Á., Henderson, G. P., Jensen, G. J., & Mekalanos, J. (2012). Type VI secretion requires a dynamic contractile phage tail-like structure. *Nature*, *483*(7388), 182-186.
17. Behzadi, P., & Behzadi, E. (2011). A study on apoptosis inducing effects of UVB irradiation in Pseudomonas aeruginosa. *Roum Arch Microbiol Immunol*, *70*(2), 74-77.
18. Behzadi, P., & Behzadi, E. (2008). The microbial agents of urinary tract infections at central laboratory of Dr. Shariati Hospital, Tehran, Iran. *Turk Klin Tip Bilim*, *28*(4), 445.
19. Berni, B., Soscia, C., Djermoun, S., Ize, B., & Bleves, S. (2019). A type VI secretion system trans-kingdom effector is required for the delivery of a novel antibacterial toxin in Pseudomonas aeruginosa. *Frontiers in Microbiology*, *10*, 1218.
20. Billings, N., Ramirez Millan, M., Caldara, M., Rusconi, R., Tarasova, Y., Stocker, R., & Ribbeck, K. (2013). The extracellular matrix component Psl provides fast-acting antibiotic defense in Pseudomonas aeruginosa biofilms. *PLoS pathogens*, *9*(8), e1003526.
21. Bingle, L. E., Bailey, C. M., & Pallen, M. J. (2008). Type VI secretion: a beginner's guide. *Current opinion in microbiology*, *11*(1), 3-8.
22. Christie, P. J., Atmakuri, K., Krishnamoorthy, V., Jakubowski, S., & Cascales, E. (2005). Biogenesis, architecture, and function of bacterial type IV secretion systems. *Annu. Rev. Microbiol.*, *59*, 451-485.

23. Bjarnsholt, T., Tolker-Nielsen, T., Høiby, N., & Givskov, M. (2010). Interference of Pseudomonas aeruginosa signalling and biofilm formation for infection control. *Expert reviews in molecular medicine*, *12*, e11.
24. Blanc, D. S., Francioli, P., & Zanetti, G. (2007). Molecular epidemiology of Pseudomonas aeruginosa in the intensive care units–a review. *The open microbiology journal*, *1*, 8.
25. Bleves, S., Viarre, V., Salacha, R., Michel, G. P., Filloux, A., & Voulhoux, R. (2010). Protein secretion systems in Pseudomonas aeruginosa: A wealth of pathogenic weapons. *International Journal of Medical Microbiology*, *300*(8), 534-543.
26. Bonomo, R. A., & Szabo, D. (2006). Mechanisms of multidrug resistance in Acinetobacter species and Pseudomonas aeruginosa. *Clinical infectious diseases*, *43*(Supplement_2), S49-S56.
27. Botelho, J., Grosso, F., & Peixe, L. (2019). Antibiotic resistance in Pseudomonas aeruginosa–Mechanisms, epidemiology and evolution. *Drug resistance updates*, *44*, 100640.
28. Brinkman, F. S., Bains, M., & Hancock, R. E. (2000). The amino terminus of Pseudomonas aeruginosa outer membrane protein OprF forms channels in lipid bilayer membranes: correlation with a three-dimensional model. *Journal of Bacteriology*, *182*(18), 5251-5255.
29. Brown, V. I., & Lowbury, E. J. L. (1965). Use of an improved cetrimide agar medium and other culture methods for Pseudomonas aeruginosa. *Journal of clinical pathology*, *18*(6), 752-756.
30. Brüggemann, H., Migliorini, L. B., Sales, R. O. D., Koga, P. C. M., Souza, A. V. D., Jensen, A., ... & Severino, P. (2018). Comparative genomics of nonoutbreak Pseudomonas aeruginosa strains underlines genome plasticity and geographic relatedness of the global clone ST235. *Genome Biology and Evolution*, *10*(7), 1852-1857.
31. Brutinel, E. D., Vakulskas, C. A., Brady, K. M., & Yahr, T. L. (2008). Characterization of ExsA and of ExsA-dependent promoters required for expression of the Pseudomonas aeruginosa type III secretion system. *Molecular microbiology*, *68*(3), 657-671.
32. Buhl, M., Peter, S., & Willmann, M. (2015). Prevalence and risk factors associated with colonization and infection of extensively drug-resistant Pseudomonas aeruginosa: a systematic review. *Expert review of anti-infective therapy*, *13*(9), 1159-1170.
33. Byrd, M. S., Sadovskaya, I., Vinogradov, E., Lu, H., Sprinkle, A. B., Richardson, S. H., & Wozniak, D. J. (2009). Genetic and biochemical analyses of the Pseudomonas aeruginosa Psl exopolysaccharide reveal overlapping roles for polysaccharide synthesis enzymes in Psl and LPS production. *Molecular microbiology*, *73*(4), 622-638.
34. Cao, H., Krishnan, G., Goumnerov, B., Tsongalis, J., Tompkins, R., & Rahme, L. G. (2001). A quorum sensing-associated virulence gene of Pseudomonas aeruginosa

encodes a LysR-like transcription regulator with a unique self-regulatory mechanism. *Proceedings of the National Academy of Sciences*, *98*(25), 14613-14618.

35. Casilag, F., Lorenz, A., Krueger, J., Klawonn, F., Weiss, S., & Häussler, S. (2016). The LasB elastase of Pseudomonas aeruginosa acts in concert with alkaline protease AprA to prevent flagellin-mediated immune recognition. *Infection and immunity*, *84*(1), 162-171.
36. Cassat, J. E., & Skaar, E. P. (2013). Iron in infection and immunity. *Cell host & microbe*, *13*(5), 509-519.
37. Chakravarty, S., Melton, C. N., Bailin, A., Yahr, T. L., & Anderson, G. G. (2017). Pseudomonas aeruginosa magnesium transporter MgtE inhibits type III secretion system gene expression by stimulating rsmYZ transcription. *Journal of bacteriology*, *199*(23), 10-1128.
38. Chambers, J. R., Cherny, K. E., & Sauer, K. (2017). Susceptibility of Pseudomonas aeruginosa dispersed cells to antimicrobial agents is dependent on the dispersion cue and class of the antimicrobial agent used. *Antimicrobial agents and chemotherapy*, *61*(12), 10-1128.
39. Chen, L., Zou, Y., Kronfl, A. A., & Wu, Y. (2020). Type VI secretion system of Pseudomonas aeruginosa is associated with biofilm formation but not environmental adaptation. *Microbiologyopen*, *9*(3), e991.
40. Chen, L., Zou, Y., She, P., & Wu, Y. (2015). Composition, function, and regulation of T6SS in Pseudomonas aeruginosa. *Microbiological research*, *172*, 19-25.
41. Cianciotto, N. P. (2005). Type II secretion: a protein secretion system for all seasons. *Trends in microbiology*, *13*(12), 581-588.
42. Ciofu, O., Fussing, V., Bagge, N., Koch, C., & Høiby, N. (2001). Characterization of paired mucoid/non-mucoid Pseudomonas aeruginosa isolates from Danish cystic fibrosis patients: antibiotic resistance, β-lactamase activity and RiboPrinting. *Journal of antimicrobial chemotherapy*, *48*(3), 391-396.
43. Ciofu, O., Tolker-Nielsen, T., Jensen, P. Ø., Wang, H., & Høiby, N. (2015). Antimicrobial resistance, respiratory tract infections and role of biofilms in lung infections in cystic fibrosis patients. *Advanced drug delivery reviews*, *85*, 7-23.
44. Coburn, B., Sekirov, I., & Finlay, B. B. (2007). Type III secretion systems and disease. *Clinical microbiology reviews*, *20*(4), 535-549.
45. Colvin, K. M., Alnabelseya, N., Baker, P., Whitney, J. C., Howell, P. L., & Parsek, M. R. (2013). PelA deacetylase activity is required for Pel polysaccharide synthesis in Pseudomonas aeruginosa. *Journal of bacteriology*, *195*(10), 2329-2339.
46. Colvin, K. M., Gordon, V. D., Murakami, K., Borlee, B. R., Wozniak, D. J., Wong, G. C., & Parsek, M. R. (2011). The pel polysaccharide can serve a structural and protective role in the biofilm matrix of Pseudomonas aeruginosa. *PLoS pathogens*, *7*(1), e1001264.
47. Colvin, K. M., Irie, Y., Tart, C. S., Urbano, R., Whitney, J. C., Ryder, C., ... & Parsek, M. R. (2012). The Pel and Psl polysaccharides provide Pseudomonas aeruginosa

structural redundancy within the biofilm matrix. *Environmental microbiology*, *14*(8), 1913-1928.

48. Costa, T. R., Felisberto-Rodrigues, C., Meir, A., Prevost, M. S., Redzej, A., Trokter, M., & Waksman, G. (2015). Secretion systems in Gram-negative bacteria: structural and mechanistic insights. *Nature Reviews Microbiology*, *13*(6), 343-359.
49. Passos da Silva, D., Matwichuk, M. L., Townsend, D. O., Reichhardt, C., Lamba, D., Wozniak, D. J., & Parsek, M. R. (2019). The Pseudomonas aeruginosa lectin LecB binds to the exopolysaccharide Psl and stabilizes the biofilm matrix. *Nature communications*, *10*(1), 2183.
50. Das, T., Kutty, S. K., Kumar, N., & Manefield, M. (2013). Pyocyanin facilitates extracellular DNA binding to Pseudomonas aeruginosa influencing cell surface properties and aggregation. *PloS one*, *8*(3), e58299.
51. Dasgupta, N., Lykken, G. L., Wolfgang, M. C., & Yahr, T. L. (2004). A novel anti-anti-activator mechanism regulates expression of the Pseudomonas aeruginosa type III secretion system. *Molecular microbiology*, *53*(1), 297-308.
52. Davies, D. G., Parsek, M. R., Pearson, J. P., Iglewski, B. H., Costerton, J. W., & Greenberg, E. P. (1998). The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science*, *280*(5361), 295-298.
53. De Kievit, T. R., & Iglewski, B. H. (2000). Bacterial quorum sensing in pathogenic relationships. *Infection and immunity*, *68*(9), 4839-4849.
54. De Sousa, T., Hébraud, M., Dapkevicius, M. L. E., Maltez, L., Pereira, J. E., Capita, R., & Poeta, P. (2021). Genomic and Metabolic Characteristics of the Pathogenicity in Pseudomonas aeruginosa. *International journal of molecular sciences*, *22*(23), 12892.
55. Delepelaire, P. (2004). Type I secretion in gram-negative bacteria. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1694*(1-3), 149-161.
56. Diggle, S. P., Stacey, R. E., Dodd, C., Cámara, M., Williams, P., & Winzer, K. (2006). The galactophilic lectin, LecA, contributes to biofilm development in Pseudomonas aeruginosa. *Environmental microbiology*, *8*(6), 1095-1104.
57. Driscoll, J. A., Brody, S. L., & Kollef, M. H. (2007). The epidemiology, pathogenesis and treatment of Pseudomonas aeruginosa infections. *Drugs*, *67*, 351-368.
58. Dufour, D., Leung, V., & Lévesque, C. M. (2010). Bacterial biofilm: structure, function, and antimicrobial resistance. *Endodontic Topics*, *22*(1), 2-16.
59. El-Fouly, M. Z., Sharaf, A. M., Shahin, A. A. M., El-Bialy, H. A., & Omara, A. M. A. (2014). Biosynthesis of pyocyanin pigment by Pseudomonas aeruginosa. *Journal of Radiation Research and Applied Sciences*, *10*(7), 1-13.
60. El-Halfawy, O. M., & Valvano, M. A. (2015). Antimicrobial heteroresistance: an emerging field in need of clarity. *Clinical microbiology reviews*, *28*(1), 191-207.
61. Eren, E., Parkin, J., Adelanwa, A., Cheneke, B., Movileanu, L., Khalid, S., & Van Den Berg, B. (2013). Toward understanding the outer membrane uptake of small molecules by Pseudomonas aeruginosa. *Journal of Biological Chemistry*, *288*(17), 12042-12053.

62. Ernst, R. K., Moskowitz, S. M., Emerson, J. C., Kraig, G. M., Adams, K. N., Harvey, M. D., ... & Miller, S. I. (2007). Unique lipid A modifications in Pseudomonas aeruginosa isolated from the airways of patients with cystic fibrosis. *The Journal of infectious diseases*, *196*(7), 1088-1092.
63. Ernst, R. K., Moskowitz, S. M., Emerson, J. C., Kraig, G. M., Adams, K. N., Harvey, M. D., ... & Miller, S. I. (2007). Unique lipid A modifications in Pseudomonas aeruginosa isolated from the airways of patients with cystic fibrosis. *The Journal of infectious diseases*, *196*(7), 1088-1092.
64. Filloux, A. (2011). Protein secretion systems in Pseudomonas aeruginosa: an essay on diversity, evolution, and function. *Frontiers in microbiology*, *2*, 155.
65. Fisher, J. F., & Mobashery, S. (2014). The sentinel role of peptidoglycan recycling in the β-lactam resistance of the Gram-negative Enterobacteriaceae and Pseudomonas aeruginosa. *Bioorganic chemistry*, *56*, 41-48.
66. Fleiszig, S. M., & Evans, D. J. (2002). The pathogenesis of bacterial keratitis: studies with Pseudomonas aeruginosa. *Clinical and Experimental Optometry*, *85*(5), 271-278.
67. Flemming, H. C., & Wingender, J. (2010). The biofilm matrix. *Nature reviews microbiology*, *8*(9), 623-633.
68. Folkesson, A., Jelsbak, L., Yang, L., Johansen, H. K., Ciofu, O., Høiby, N., & Molin, S. (2012). Adaptation of Pseudomonas aeruginosa to the cystic fibrosis airway: an evolutionary perspective. *Nature Reviews Microbiology*, *10*(12), 841-851.
69. Francis, M. S., Wolf-Watz, H., & Forsberg, Å. (2002). Regulation of type III secretion systems. *Current opinion in microbiology*, *5*(2), 166-172.
70. Frank, D. W. (1997). The exoenzyme S regulon of Pseudomonas aeruginosa. *Molecular microbiology*, *26*(4), 621-629.
71. Franklin, M. J., Nivens, D. E., Weadge, J. T., & Howell, P. L. (2011). Biosynthesis of the Pseudomonas aeruginosa extracellular polysaccharides, alginate, Pel, and Psl. *Frontiers in microbiology*, *2*, 167.
72. Friedman, L., & Kolter, R. (2004). Genes involved in matrix formation in Pseudomonas aeruginosa PA14 biofilms. *Molecular microbiology*, *51*(3), 675-690.
73. Gajdács, M., Burián, K., & Terhes, G. (2019). Resistance levels and epidemiology of non-fermenting gram-negative bacteria in urinary tract infections of inpatients and outpatients (RENFUTI): a 10-year epidemiological snapshot. *Antibiotics*, *8*(3), 143.
74. Garai, P., Berry, L., Moussouni, M., Bleves, S., & Blanc-Potard, A. B. (2019). Killing from the inside: Intracellular role of T3SS in the fate of Pseudomonas aeruginosa within macrophages revealed by mgtC and oprF mutants. *PLoS pathogens*, *15*(6), e1007812.
75. Gellatly, S. L., Needham, B., Madera, L., Trent, M. S., & Hancock, R. E. (2012). The Pseudomonas aeruginosa PhoP-PhoQ two-component regulatory system is induced upon interaction with epithelial cells and controls cytotoxicity and inflammation. *Infection and immunity*, *80*(9), 3122-3131.

76. Ghafoor, A., Hay, I. D., & Rehm, B. H. (2011). Role of exopolysaccharides in Pseudomonas aeruginosa biofilm formation and architecture. *Applied and environmental microbiology*, *77*(15), 5238-5246.
77. Giamarellou, H. (2002). Prescribing guidelines for severe Pseudomonas infections. *Journal of Antimicrobial Chemotherapy*, *49*(2), 229-233.
78. Gilleland, L. B., Gilleland, H. E., Gibson, J. A., & Champlin, F. R. (1989). Adaptive resistance to aminoglycoside antibiotics in Pseudomonas aeruginosa. *Journal of medical microbiology*, *29*(1), 41-50.
79. Giwercman, B., Lambert, P. A., Rosdahl, V. T., Shand, G. H., & Heiby, N. (1990). Rapid emergence of resistance in Pseudomonas aeruginosa in cystic fibrosis patients due to in-vivo selection of stable partially derepressed β-lactamase producing strains. *Journal of Antimicrobial Chemotherapy*, *26*(2), 247-259.
80. Giwercman, B., Meyer, C., Lambert, P. A., Reinert, C., & Høiby, N. (1992). High-level beta-lactamase activity in sputum samples from cystic fibrosis patients during antipseudomonal treatment. *Antimicrobial agents and chemotherapy*, *36*(1), 71-76.
81. Gloag, E. S., German, G. K., Stoodley, P., & Wozniak, D. J. (2018). Viscoelastic properties of Pseudomonas aeruginosa variant biofilms. *Scientific reports*, *8*(1), 9691.
82. Goodman, A. L., Kulasekara, B., Rietsch, A., Boyd, D., Smith, R. S., & Lory, S. (2004). A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in Pseudomonas aeruginosa. *Developmental cell*, *7*(5), 745-754.
83. Gurney, J., Pradier, L., Griffin, J. S., Gougat-Barbera, C., Chan, B. K., Turner, P. E., ... & Hochberg, M. E. (2020). Phage steering of antibiotic-resistance evolution in the bacterial pathogen, Pseudomonas aeruginosa. *Evolution, medicine, and public health*, *2020*(1), 148-157.
84. Guttenplan, S. B., & Kearns, D. B. (2013). Regulation of flagellar motility during biofilm formation. *FEMS microbiology reviews*, *37*(6), 849-871.
85. Vala, M. H., Hallajzadeh, M., Hashemi, A., Goudarzi, H., Tarhani, M., Tabrizi, M. S., & Bazmi, F. (2014). Detection of Ambler class A, B and D ß-lactamases among Pseudomonas aeruginosa and Acinetobacter baumannii clinical isolates from burn patients. *Annals of Burns and Fire Disasters*, *27*(1), 8.
86. Hall, S., McDermott, C., Anoopkumar-Dukie, S., McFarland, A. J., Forbes, A., Perkins, A. V., ... & Grant, G. D. (2016). Cellular effects of pyocyanin, a secreted virulence factor of Pseudomonas aeruginosa. *Toxins*, *8*(8), 236.
87. Hall-Stoodley, L., & Stoodley, P. (2009). Evolving concepts in biofilm infections. *Cellular microbiology*, *11*(7), 1034-1043.
88. Hancock, R. E., Mutharia, L. M., Chan, L., Darveau, R. P., Speert, D. P., & Pier, G. (1983). Pseudomonas aeruginosa isolates from patients with cystic fibrosis: a class of serum-sensitive, nontypable strains deficient in lipopolysaccharide O side chains. *Infection and immunity*, *42*(1), 170-177.

89. Hancock, R. E. (1998). Resistance mechanisms in Pseudomonas aeruginosa and other nonfermentative gram-negative bacteria. *Clinical Infectious Diseases*, *27*(Supplement_1), S93-S99.
90. Harshey, R. M. (2003). Bacterial motility on a surface: many ways to a common goal. *Annual Reviews in Microbiology*, *57*(1), 249-273.
91. Hay, I. D., Ur Rehman, Z., Ghafoor, A., & Rehm, B. H. (2010). Bacterial biosynthesis of alginates. *Journal of Chemical Technology & Biotechnology*, *85*(6), 752-759.
92. Hay, I. D., Rehman, Z. U., Moradali, M. F., Wang, Y., & Rehm, B. H. (2013). Microbial alginate production, modification and its applications. *Microbial biotechnology*, *6*(6), 637-650.
93. Hobden, J. A. (2002). Pseudomonas aeruginosa proteases and corneal virulence. *DNA and cell biology*, *21*(5-6), 391-396.
94. Hogardt, M., & Heesemann, J. (2010). Adaptation of Pseudomonas aeruginosa during persistence in the cystic fibrosis lung. *International Journal of Medical Microbiology*, *300*(8), 557-562.
95. Hogardt, M., & Heesemann, J. (2012). Microevolution of Pseudomonas aeruginosa to a chronic pathogen of the cystic fibrosis lung. *Between pathogenicity and commensalism*, 91-118.
96. Høiby, N. (2011). Recent advances in the treatment of Pseudomonas aeruginosa infections in cystic fibrosis. *BMC medicine*, *9*, 1-7.
97. Høiby, N., Ciofu, O., Johansen, H. K., Song, Z. J., Moser, C., Jensen, P. Ø., ... & Bjarnsholt, T. (2011). The clinical impact of bacterial biofilms. *International journal of oral science*, *3*(2), 55-65.
98. Hood, R. D., Singh, P., Hsu, F., Güvener, T., Carl, M. A., Trinidad, R. R., & Mougous, J. D. (2010). A type VI secretion system of Pseudomonas aeruginosa targets a toxin to bacteria. *Cell host & microbe*, *7*(1), 25-37.
99. Huang, H., Shao, X., Xie, Y., Wang, T., Zhang, Y., Wang, X., & Deng, X. (2019). An integrated genomic regulatory network of virulence-related transcriptional factors in Pseudomonas aeruginosa. *Nature communications*, *10*(1), 2931.
100. Irie, Y., Borlee, B. R., O'Connor, J. R., Hill, P. J., Harwood, C. S., Wozniak, D. J., & Parsek, M. R. (2012). Self-produced exopolysaccharide is a signal that stimulates biofilm formation in Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences*, *109*(50), 20632-20636.
101. Irie, Y., Roberts, A. E., Kragh, K. N., Gordon, V. D., Hutchison, J., Allen, R. J., ... & Diggle, S. P. (2017). The Pseudomonas aeruginosa PSL polysaccharide is a social but noncheatable trait in biofilms. *MBio*, *8*(3), 10-1128.
102. Iyer, R., Williams, C., & Miller, C. (2003). Arginine-agmatine antiporter in extreme acid resistance in Escherichia coli. *Journal of bacteriology*, *185*(22), 6556-6561.

103. Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., & Kamil, M. A. (2018). Bacterial biofilm and associated infections. *Journal of the chinese medical association*, *81*(1), 7-11.
104. Jansen, G., Mahrt, N., Tueffers, L., Barbosa, C., Harjes, M., Adolph, G., & Schulenburg, H. (2016). Association between clinical antibiotic resistance and susceptibility of Pseudomonas in the cystic fibrosis lung. *Evolution, medicine, and public health*, *2016*(1), 182-194.
105. Jennings, L. K., Storek, K. M., Ledvina, H. E., Coulon, C., Marmont, L. S., Sadovskaya, I., ... & Parsek, M. R. (2015). Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the Pseudomonas aeruginosa biofilm matrix. *Proceedings of the National Academy of Sciences*, *112*(36), 11353-11358.
106. Jin, Y., Yang, H., Qiao, M., & Jin, S. (2011). MexT regulates the type III secretion system through MexS and PtrC in Pseudomonas aeruginosa. *Journal of bacteriology*, *193*(2), 399-410.
107. Jones, C. J., & Wozniak, D. J. (2017). Psl produced by mucoid Pseudomonas aeruginosa contributes to the establishment of biofilms and immune evasion. *MBio*, *8*(3), 10-1128.
108. Juhas, M., Crook, D. W., & Hood, D. W. (2008). Type IV secretion systems: tools of bacterial horizontal gene transfer and virulence. *Cellular microbiology*, *10*(12), 2377-2386.
109. Kampf, G. (2016). Acquired resistance to chlorhexidine–is it time to establish an 'antiseptic stewardship'initiative?. *Journal of Hospital Infection*, *94*(3), 213-227.
110. Kanamaru, S. (2009). Structural similarity of tailed phages and pathogenic bacterial secretion systems. *Proceedings of the National Academy of Sciences*, *106*(11), 4067-4068.
111. Kielhofner, M., Atmar, R. L., Hamill, R. J., & Musher, D. M. (1992). Life-threatening Pseudomonas aeruginosa infections in patients with human immunodeficiency virus infection. *Clinical infectious diseases*, *14*(2), 403-411.
112. King, J. D., Kocíncová, D., Westman, E. L., & Lam, J. S. (2009). Lipopolysaccharide biosynthesis in Pseudomonas aeruginosa. *Innate immunity*, *15*(5), 261-312.
113. Kipnis, E., Sawa, T., & Wiener-Kronish, J. (2006). Targeting mechanisms of Pseudomonas aeruginosa pathogenesis. *Medecine et maladies infectieuses*, *36*(2), 78-91.
114. Klockgether, J., Cramer, N., Wiehlmann, L., Davenport, C. F., & Tümmler, B. (2011). Pseudomonas aeruginosa genomic structure and diversity. *Frontiers in microbiology*, *2*, 150.
115. Klockgether, J., & Tümmler, B. (2017). Recent advances in understanding Pseudomonas aeruginosa as a pathogen. *F1000Research*, *6*.

116. Korotkov, K. V., Sandkvist, M., & Hol, W. G. (2012). The type II secretion system: biogenesis, molecular architecture and mechanism. *Nature Reviews Microbiology*, *10*(5), 336-351.
117. Korten, V., Akalin, H., Kocagoz, S., Kocazeybek, B. S., Ozturk, R., Vahaboglu, H., & Ozinel, M. A. (2001). Clinical importance of extended-spectrum beta-lactamase (PER-1-type)-producing Acinetobacter spp. and Pseudomonas aeruginosa strains.
118. Kuang, Z., Hao, Y., Walling, B. E., Jeffries, J. L., Ohman, D. E., & Lau, G. W. (2011). Pseudomonas aeruginosa elastase provides an escape from phagocytosis by degrading the pulmonary surfactant protein-A. *PloS one*, *6*(11), e27091.
119. Das, T., Kutty, S. K., Tavallaie, R., Ibugo, A. I., Panchompoo, J., Sehar, S., & Manefield, M. (2015). Phenazine virulence factor binding to extracellular DNA is important for Pseudomonas aeruginosa biofilm formation. *Scientific Reports*, *5*(1), 8398.
120. Kumarage, J., Khonyongwa, K., Khan, A., Desai, N., Hoffman, P., & Taori, S. K. (2019). Transmission of multi-drug resistant Pseudomonas aeruginosa between two flexible ureteroscopes and an outbreak of urinary tract infection: the fragility of endoscope decontamination. *Journal of Hospital Infection*, *102*(1), 89-94.
121. Kwon, D. H., & Lu, C. D. (2006). Polyamines induce resistance to cationic peptide, aminoglycoside, and quinolone antibiotics in Pseudomonas aeruginosa PAO1. *Antimicrobial agents and chemotherapy*, *50*(5), 1615-1622.
122. Laarman, A. J., Bardoel, B. W., Ruyken, M., Fernie, J., Milder, F. J., van Strijp, J. A., & Rooijakkers, S. H. (2012). Pseudomonas aeruginosa alkaline protease blocks complement activation via the classical and lectin pathways. *The Journal of Immunology*, *188*(1), 386-393.
123. Lam, J. S., Taylor, V. L., Islam, S. T., Hao, Y., & Kocíncová, D. (2011). Genetic and functional diversity of Pseudomonas aeruginosa lipopolysaccharide. *Frontiers in microbiology*, *2*, 118.
124. Clark, S. T., Diaz Caballero, J., Cheang, M., Coburn, B., Wang, P. W., Donaldson, S. L., & Hwang, D. M. (2015). Phenotypic diversity within a Pseudomonas aeruginosa population infecting an adult with cystic fibrosis. *Scientific reports*, *5*(1), 10932.
125. Lawley, T. D., Klimke, W. A., Gubbins, M. J., & Frost, L. S. (2003). F factor conjugation is a true type IV secretion system. *FEMS microbiology letters*, *224*(1), 1-15.
126. Lee, B., Schjerling, C. K., Kirkby, N., Hoffmann, N., Borup, R., Molin, S., ... & Ciofu, O. (2011). Mucoid Pseudomonas aeruginosa isolates maintain the biofilm formation capacity and the gene expression profiles during the chronic lung infection of CF patients. *Apmis*, *119*(4-5), 263-274.
127. Lee, J. H., Kim, Y. G., Ryu, S. Y., Cho, M. H., & Lee, J. (2014). Ginkgolic acids and Ginkgo biloba extract inhibit Escherichia coli O157: H7 and Staphylococcus aureus biofilm formation. *International journal of food microbiology*, *174*, 47-55.

128. Lee, J., & Zhang, L. (2015). The hierarchy quorum sensing network in Pseudomonas aeruginosa. *Protein & cell*, *6*(1), 26-41.
129. Lee, P. A., Tullman-Ercek, D., & Georgiou, G. (2006). The bacterial twin-arginine translocation pathway. *Annu. Rev. Microbiol.*, *60*, 373-395.
130. Lesic, Á., Starkey, Á., He, J., Hazan, R., & Rahme, L. G. (2009). Quorum sensing differentially regulates Pseudomonas aeruginosa type VI secretion locus I and homologous loci II and III, which are required for pathogenesis. *Microbiology*, *155*(Pt 9), 2845.
131. Lesic, Á., Starkey, Á., He, J., Hazan, R., & Rahme, L. G. (2009). Quorum sensing differentially regulates Pseudomonas aeruginosa type VI secretion locus I and homologous loci II and III, which are required for pathogenesis. *Microbiology*, *155*(Pt 9), 2845.
132. Lewis, K. I. M. (2001). Riddle of biofilm resistance. *Antimicrobial agents and chemotherapy*, *45*(4), 999-1007.
133. Li, M., Long, Y., Liu, Y., Liu, Y., Chen, R., Shi, J., ... & Wu, W. (2016). HigB of Pseudomonas aeruginosa enhances killing of phagocytes by up-regulating the type III secretion system in ciprofloxacin induced persister cells. *Frontiers in cellular and infection microbiology*, *6*, 125.
134. Li, X. Z., Plésiat, P., & Nikaido, H. (2015). The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clinical microbiology reviews*, *28*(2), 337-418.
135. Lieleg, O., Caldara, M., Baumgärtel, R., & Ribbeck, K. (2011). Mechanical robustness of Pseudomonas aeruginosa biofilms. *Soft matter*, *7*(7), 3307-3314.
136. Lister, P. D., Wolter, D. J., & Hanson, N. D. (2009). Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical microbiology reviews*, *22*(4), 582-610.
137. Livermore, D. M. (2001). Of Pseudomonas, porins, pumps and carbapenems. *Journal of Antimicrobial Chemotherapy*, *47*(3), 247-250.
138. Ma, L., Conover, M., Lu, H., Parsek, M. R., Bayles, K., & Wozniak, D. J. (2009). Assembly and development of the Pseudomonas aeruginosa biofilm matrix. *PLoS pathogens*, *5*(3), e1000354.
139. Ma, L., Wang, S., Wang, D., Parsek, M. R., & Wozniak, D. J. (2012). The roles of biofilm matrix polysaccharide Psl in mucoid Pseudomonas aeruginosa biofilms. *FEMS Immunology & Medical Microbiology*, *65*(2), 377-380.
140. Ma, L., Wang, J., Wang, S., Anderson, E. M., Lam, J. S., Parsek, M. R., & Wozniak, D. J. (2012). Synthesis of multiple Pseudomonas aeruginosa biofilm matrix exopolysaccharides is post-transcriptionally regulated. *Environmental microbiology*, *14*(8), 1995-2005.

141. Ma, Q., Zhai, Y., Schneider, J. C., Ramseier, T. M., & Saier Jr, M. H. (2003). Protein secretion systems of Pseudomonas aeruginosa and P. fluorescens. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1611*(1-2), 223-233.

142. MacLeod, D. L., Nelson, L. E., Shawar, R. M., Lin, B. B., Lockwood, L. G., Dirks, J. E., ... & Garber, R. L. (2000). Aminoglycoside-resistance mechanisms for cystic fibrosis Pseudomonas aeruginosa isolates are unchanged by long-term, intermittent, inhaled tobramycin treatment. *The Journal of infectious diseases*, *181*(3), 1180-1184.

143. Mah, T. F., Pitts, B., Pellock, B., Walker, G. C., Stewart, P. S., & O'Toole, G. A. (2003). A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance. *Nature*, *426*(6964), 306-310.

144. Maiti, S. N., Phillips, O. A., Micetich, R. G., & Livermore, D. M. (1998). Beta-lactamase inhibitors: agents to overcome bacterial resistance. *Current medicinal chemistry*, *5*(6), 441-456.

145. Mapipa, Q., Digban, T. O., Nnolim, N. E., & Nwodo, U. U. (2021). Antibiogram profile and virulence signatures of Pseudomonas aeruginosa isolates recovered from selected agrestic hospital effluents. *Scientific Reports*, *11*(1), 11800.

146. Mariencheck, W. I., Alcorn, J. F., Palmer, S. M., & Wright, J. R. (2003). Pseudomonas aeruginosa elastase degrades surfactant proteins A and D. *American journal of respiratory cell and molecular biology*, *28*(4), 528-537.

147. Marsden, A. E., Intile, P. J., Schulmeyer, K. H., Simmons-Patterson, E. R., Urbanowski, M. L., Wolfgang, M. C., & Yahr, T. L. (2016). Vfr directly activates exsA transcription to regulate expression of the Pseudomonas aeruginosa type III secretion system. *Journal of bacteriology*, *198*(9), 1442-1450.

148. Mashburn, L. M., & Whiteley, M. (2005). Membrane vesicles traffic signals and facilitate group activities in a prokaryote. *Nature*, *437*(7057), 422-425.

149. Matsumoto, K. (2004). Role of bacterial proteases in pseudomonal and serratial keratitis.

150. Maurice, N. M., Bedi, B., & Sadikot, R. T. (2018). Pseudomonas aeruginosa biofilms: host response and clinical implications in lung infections. *American journal of respiratory cell and molecular biology*, *58*(4), 428-439.

151. McCaw, M. L., Lykken, G. L., Singh, P. K., & Yahr, T. L. (2002). ExsD is a negative regulator of the Pseudomonas aeruginosa type III secretion regulon. *Molecular microbiology*, *46*(4), 1123-1133.

152. McKnight, S. L., Iglewski, B. H., & Pesci, E. C. (2000). The Pseudomonas quinolone signal regulates rhl quorum sensing in Pseudomonas aeruginosa. *Journal of bacteriology*, *182*(10), 2702-2708.

153. Meuskens, I., Saragliadis, A., Leo, J. C., & Linke, D. (2019). Type V secretion systems: an overview of passenger domain functions. *Frontiers in microbiology*, *10*, 1163.

154. Meyer, J. M., Neely, A., Stintzi, A., Georges, C., & Holder, I. A. (1996). Pyoverdin is essential for virulence of Pseudomonas aeruginosa. *Infection and immunity*, *64*(2), 518-523.

155. Miao, E. A., Andersen-Nissen, E., Warren, S. E., & Aderem, A. (2007, September). TLR5 and Ipaf: dual sensors of bacterial flagellin in the innate immune system. In *Seminars in immunopathology* (Vol. 29, pp. 275-288). Springer-Verlag.

156. Migula, W. (1900). *System der bakterien: Handbuch der morphologie, entwicklungsgeschichte und systematik der bakterien* (Vol. 2). Fischer.

157. Miller, M. B., & Bassler, B. L. (2001). Quorum sensing in bacteria. *Annual Reviews in Microbiology*, *55*(1), 165-199.

158. Mishra, M., Byrd, M. S., Sergeant, S., Azad, A. K., Parsek, M. R., McPhail, L., ... & Wozniak, D. J. (2012). Pseudomonas aeruginosa Psl polysaccharide reduces neutrophil phagocytosis and the oxidative response by limiting complement-mediated opsonization. *Cellular microbiology*, *14*(1), 95-106.

159. Mukherjee, S., & Bassler, B. L. (2019). Bacterial quorum sensing in complex and dynamically changing environments. *Nature Reviews Microbiology*, *17*(6), 371-382.

160. Mulcahy, H., Charron-Mazenod, L., & Lewenza, S. (2010). Pseudomonas aeruginosa produces an extracellular deoxyribonuclease that is required for utilization of DNA as a nutrient source. *Environmental microbiology*, *12*(6), 1621-1629.

161. Nanvazadeh, F., Khosravi, A. D., Zolfaghari, M. R., & Parhizgari, N. (2013). Genotyping of Pseudomonas aeruginosa strains isolated from burn patients by RAPD-PCR. *Burns*, *39*(7), 1409-1413.

162. Naskar, S., Hohl, M., Tassinari, M., & Low, H. H. (2021). The structure and mechanism of the bacterial type II secretion system. *Molecular Microbiology*, *115*(3), 412-424.

163. Nichols, W. W., Dorrington, S. M., Slack, M. P., & Walmsley, H. L. (1988). Inhibition of tobramycin diffusion by binding to alginate. *Antimicrobial agents and chemotherapy*, *32*(4), 518-523.

164. Nikaido, H. (1998). Multiple antibiotic resistance and efflux. *Current opinion in microbiology*, *1*(5), 516-523.

165. Nivaskumar, M., & Francetic, O. (2014). Type II secretion system: a magic beanstalk or a protein escalator. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1843*(8), 1568-1577.

166. Normark, B. H., & Normark, S. (2002). Evolution and spread of antibiotic resistance. *Journal of internal medicine*, *252*(2), 91-106.

167. O'May, C., & Tufenkji, N. (2011). The swarming motility of Pseudomonas aeruginosa is blocked by cranberry proanthocyanidins and other tannin-containing materials. *Applied and environmental microbiology*, *77*(9), 3061-3067.

168. Oglesby, A. G., Farrow, J. M., Lee, J. H., Tomaras, A. P., Greenberg, E. P., Pesci, E. C., & Vasil, M. L. (2008). The influence of iron on Pseudomonas aeruginosa

physiology: a regulatory link between iron and quorum sensing. *Journal of Biological Chemistry*, *283*(23), 15558-15567.

169. Oliver, A., Mulet, X., López-Causapé, C., & Juan, C. (2015). The increasing threat of Pseudomonas aeruginosa high-risk clones. *Drug Resistance Updates*, *21*, 41-59.
170. Ostroff, R. M., Vasil, A. I., & Vasil, M. L. (1990). Molecular comparison of a nonhemolytic and a hemolytic phospholipase C from Pseudomonas aeruginosa. *Journal of Bacteriology*, *172*(10), 5915-5923.
171. Ozer, E., Yaniv, K., Chetrit, E., Boyarski, A., Meijler, M. M., Berkovich, R., ... & Alfonta, L. (2021). An inside look at a biofilm: Pseudomonas aeruginosa flagella biotracking. *Science advances*, *7*(24), eabg8581.
172. Pachori, P., Gothalwal, R., & Gandhi, P. (2019). Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. *Genes & diseases*, *6*(2), 109-119.
173. Palleroni, N. J. (2010). The pseudomonas story. *Environmental microbiology*, *12*(6), 1377-1383.
174. Palmer, G. C., Palmer, K. L., Jorth, P. A., & Whiteley, M. (2010). Characterization of the Pseudomonas aeruginosa transcriptional response to phenylalanine and tyrosine. *Journal of bacteriology*, *192*(11), 2722-2728.
175. Pamp, S. J., & Tolker-Nielsen, T. (2007). Multiple roles of biosurfactants in structural biofilm development by Pseudomonas aeruginosa. *Journal of bacteriology*, *189*(6), 2531-2539.
176. Park, W. S., Lee, J., Na, G., Park, S., Seo, S. K., Choi, J. S., ... & Choi, I. W. (2022). Benzyl Isothiocyanate Attenuates Inflammasome Activation in Pseudomonas aeruginosa LPS-Stimulated THP-1 Cells and Exerts Regulation through the MAPKs/NF-κB Pathway. *International Journal of Molecular Sciences*, *23*(3), 1228.
177. Parkins, M. D., Somayaji, R., & Waters, V. J. (2018). Epidemiology, biology, and impact of clonal Pseudomonas aeruginosa infections in cystic fibrosis. *Clinical microbiology reviews*, *31*(4), 10-1128.
178. Pearson, J. P., Pesci, E. C., & Iglewski, B. H. (1997). Roles of Pseudomonas aeruginosa las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *Journal of bacteriology*, *179*(18), 5756-5767.
179. Pesci, E. C., & Iglewski, B. H. (1997). The chain of command in Pseudomonas quorum sensing. *Trends in microbiology*, *5*(4), 132-134.
180. Poole, K. (2001). Multidrug efflux pumps and antimicrobial resistance in Pseudomonas aeruginosa and related organisms. *Journal of molecular microbiology and biotechnology*, *3*(2), 255-264.
181. Poole, K. (2004). Efflux-mediated multiresistance in Gram-negative bacteria. *Clinical Microbiology and infection*, *10*(1), 12-26.
182. Pukatzki, S., Ma, A. T., Sturtevant, D., Krastins, B., Sarracino, D., Nelson, W. C., ... & Mekalanos, J. J. (2006). Identification of a conserved bacterial protein secretion

system in Vibrio cholerae using the Dictyostelium host model system. *Proceedings of the National Academy of Sciences*, *103*(5), 1528-1533.

183. Quick, J., Cumley, N., Wearn, C. M., Niebel, M., Constantinidou, C., Thomas, C. M., ... & Loman, N. J. (2014). Seeking the source of Pseudomonas aeruginosa infections in a recently opened hospital: an observational study using whole-genome sequencing. *BMJ open*, *4*(11), e006278.

184. Ramos, J. L. (Ed.). (2011). *Pseudomonas: Volume 1 Genomics, Life Style and Molecular Architecture*. Springer Science & Business Media.

185. Rampioni, G., Falcone, M., Heeb, S., Frangipani, E., Fletcher, M. P., Dubern, J. F., ... & Williams, P. (2016). Unravelling the genome-wide contributions of specific 2-alkyl-4-quinolones and PqsE to quorum sensing in Pseudomonas aeruginosa. *PLoS pathogens*, *12*(11), e1006029.

186. Rampioni, G., Leoni, L., & Williams, P. (2014). The art of antibacterial warfare: deception through interference with quorum sensing–mediated communication. *Bioorganic Chemistry*, *55*, 60-68.

187. Rasamiravaka, T., Labtani, Q., Duez, P., & El Jaziri, M. (2015). The formation of biofilms by Pseudomonas aeruginosa: a review of the natural and synthetic compounds interfering with control mechanisms. *BioMed research international*, *2015*.

188. Rasko, D. A., & Sperandio, V. (2010). Anti-virulence strategies to combat bacteria-mediated disease. *Nature reviews Drug discovery*, *9*(2), 117-128.

189. Rees, V. E., Deveson Lucas, D. S., López-Causapé, C., Huang, Y., Kotsimbos, T., Bulitta, J. B., ... & Landersdorfer, C. B. (2019). Characterization of hypermutator Pseudomonas aeruginosa isolates from patients with cystic fibrosis in Australia. *Antimicrobial agents and chemotherapy*, *63*(4), 10-1128.

190. Rehm, B. H. A., & Valla, S. (1997). Bacterial alginates: biosynthesis and applications. *Applied microbiology and biotechnology*, *48*, 281-288.

191. Ribet, D., & Cossart, P. (2015). How bacterial pathogens colonize their hosts and invade deeper tissues. *Microbes and infection*, *17*(3), 173-183.

192. Rietsch, A., Vallet-Gely, I., Dove, S. L., & Mekalanos, J. J. (2005). ExsE, a secreted regulator of type III secretion genes in Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences*, *102*(22), 8006-8011.

193. Russell, A. B., Hood, R. D., Bui, N. K., LeRoux, M., Vollmer, W., & Mougous, J. D. (2011). Type VI secretion delivers bacteriolytic effectors to target cells. *Nature*, *475*(7356), 343-347.

194. Rybtke, M., Berthelsen, J., Yang, L., Høiby, N., Givskov, M., & Tolker-Nielsen, T. (2015). The LapG protein plays a role in Pseudomonas aeruginosa biofilm formation by controlling the presence of the CdrA adhesin on the cell surface. *Microbiologyopen*, *4*(6), 917-930.

195. Ryder, C., Byrd, M., & Wozniak, D. J. (2007). Role of polysaccharides in Pseudomonas aeruginosa biofilm development. *Current opinion in microbiology*, *10*(6), 644-648.

196. Sabnis, A., Hagart, K. L., Klöckner, A., Becce, M., Evans, L. E., Furniss, R. C. D., ... & Edwards, A. M. (2021). Colistin kills bacteria by targeting lipopolysaccharide in the cytoplasmic membrane. *elife*, *10*, e65836.

197. Sadikot, R. T., Blackwell, T. S., Christman, J. W., & Prince, A. S. (2005). Pathogen–host interactions in Pseudomonas aeruginosa pneumonia. *American journal of respiratory and critical care medicine*, *171*(11), 1209-1223.

198. Sakuragi, Y., & Kolter, R. (2007). Quorum-sensing regulation of the biofilm matrix genes (pel) of Pseudomonas aeruginosa. *Journal of bacteriology*, *189*(14), 5383-5386.

199. Sana, T. G., Hachani, A., Bucior, I., Soscia, C., Garvis, S., Termine, E., ... & Bleves, S. (2012). The second type VI secretion system of Pseudomonas aeruginosa strain PAO1 is regulated by quorum sensing and Fur and modulates internalization in epithelial cells. *Journal of Biological Chemistry*, *287*(32), 27095-27105.

200. Sawa, T., Shimizu, M., Moriyama, K., & Wiener-Kronish, J. P. (2014). Association between Pseudomonas aeruginosa type III secretion, antibiotic resistance, and clinical outcome: a review. *Critical Care*, *18*(6), 1-12.

201. Schwartz, T., Jungfer, C., Heißler, S., Friedrich, F., Faubel, W., & Obst, U. (2009). Combined use of molecular biology taxonomy, Raman spectrometry, and ESEM imaging to study natural biofilms grown on filter materials at waterworks. *Chemosphere*, *77*(2), 249-257.

202. Seed, P. C., Passador, L., & Iglewski, B. H. (1995). Activation of the Pseudomonas aeruginosa lasI gene by LasR and the Pseudomonas autoinducer PAI: an autoinduction regulatory hierarchy. *Journal of bacteriology*, *177*(3), 654-659.

203. Sharma, A. K., Dhasmana, N., Dubey, N., Kumar, N., Gangwal, A., Gupta, M., & Singh, Y. (2017). Bacterial virulence factors: secreted for survival. *Indian journal of microbiology*, *57*, 1-10.

204. Shaw, E., & Wuest, W. M. (2020). Virulence attenuating combination therapy: A potential multi-target synergy approach to treat Pseudomonas aeruginosa infections in cystic fibrosis patients. *RSC Medicinal Chemistry*, *11*(3), 358-369.

205. Shen, D. K., Filopon, D., Kuhn, L., Polack, B., & Toussaint, B. (2006). PsrA is a positive transcriptional regulator of the type III secretion system in Pseudomonas aeruginosa. *Infection and immunity*, *74*(2), 1121-1129.

206. Qin, S., Xiao, W., Zhou, C., Pu, Q., Deng, X., Lan, L., ... & Wu, M. (2022). Pseudomonas aeruginosa: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal transduction and targeted therapy*, *7*(1), 199.

207. Slekovec, C., Plantin, J., Cholley, P., Thouverez, M., Talon, D., Bertrand, X., & Hocquet, D. (2012). Tracking down antibiotic-resistant Pseudomonas aeruginosa isolates in a wastewater network. *PloS one*, *7*(12), e49300.

208. Smith, E. E., Buckley, D. G., Wu, Z., Saenphimmachak, C., Hoffman, L. R., D'Argenio, D. A., & Olson, M. V. (2006). Genetic adaptation by Pseudomonas aeruginosa to the airways of cystic fibrosis patients. *Proceedings of the National Academy of Sciences*, *103*(22), 8487-8492.

209. Snyder, L. A., Loman, N. J., Faraj, L. A., Levi, K., Weinstock, G., Boswell, T. C., ... & Ala'Aldeen, D. A. (2013). Epidemiological investigation of Pseudomonas aeruginosa isolates from a six-year-long hospital outbreak using high-throughput whole genome sequencing. *Eurosurveillance*, *18*(42).

210. Soscia, C., Hachani, A., Bernadac, A., Filloux, A., & Bleves, S. (2007). Cross talk between type III secretion and flagellar assembly systems in Pseudomonas aeruginosa. *Journal of Bacteriology*, *189*(8), 3124-3132.

211. Spiers, A. J., Buckling, A., & Rainey, P. B. (2000). The causes of Pseudomonas diversity. *Microbiology*, *146*(10), 2345-2350.

212. Staudinger, B. J., Muller, J. F., Halldórsson, S., Boles, B., Angermeyer, A., Nguyen, D., ... & Singh, P. K. (2014). Conditions associated with the cystic fibrosis defect promote chronic Pseudomonas aeruginosa infection. *American journal of respiratory and critical care medicine*, *189*(7), 812-824.

213. Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., ... & Olson, M. V. (2000). Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. *Nature*, *406*(6799), 959-964.

214. Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., ... & Olson, M. V. (2000). Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. *Nature*, *406*(6799), 959-964.

215. Strateva, T., & Mitov, I. (2011). Contribution of an arsenal of virulence factors to pathogenesis of Pseudomonas aeruginosa infections. *Annals of microbiology*, *61*(4), 717-732.

216. Strempel, N., Neidig, A., Nusser, M., Geffers, R., Vieillard, J., Lesouhaitier, O., ... & Overhage, J. (2013). Human host defense peptide LL-37 stimulates virulence factor production and adaptive resistance in Pseudomonas aeruginosa. *PloS one*, *8*(12), e82240.

217. Tammam, S., Sampaleanu, L. M., Koo, J., Manoharan, K., Daubaras, M., Burrows, L. L., & Howell, P. L. (2013). PilMNOPQ from the Pseudomonas aeruginosa type IV pilus system form a transenvelope protein interaction network that interacts with PilA. *Journal of bacteriology*, *195*(10), 2126-2135.

218. Teghanemt, A., Zhang, D., Levis, E. N., Weiss, J. P., & Gioannini, T. L. (2005). Molecular basis of reduced potency of underacylated endotoxins. *The Journal of Immunology*, *175*(7), 4669-4676.

219. Thomas, S., Holland, I. B., & Schmitt, L. (2014). The type 1 secretion pathway—the hemolysin system and beyond. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1843*(8), 1629-1641.

220. Toder, D. S., Ferrell, S. J., Nezezon, J. L., Rust, L., & Iglewski, B. H. (1994). lasA and lasB genes of Pseudomonas aeruginosa: analysis of transcription and gene product activity. *Infection and immunity*, *62*(4), 1320-1327.

221. Tseng, B. S., Zhang, W., Harrison, J. J., Quach, T. P., Song, J. L., Penterman, J., ... & Parsek, M. R. (2013). The extracellular matrix protects P seudomonas aeruginosa biofilms by limiting the penetration of tobramycin. *Environmental microbiology*, *15*(10), 2865-2878.

222. Turan, N. B., Chormey, D. S., Büyükpınar, Ç., Engin, G. O., & Bakirdere, S. (2017). Quorum sensing: Little talks for an effective bacterial coordination. *TrAC Trends in Analytical Chemistry*, *91*, 1-11.

223. Urbanowski, M. L., Lykken, G. L., & Yahr, T. L. (2005). A secreted regulatory protein couples transcription to the secretory activity of the Pseudomonas aeruginosa type III secretion system. *Proceedings of the National Academy of Sciences*, *102*(28), 9930-9935.

224. Vaisvila, R., Morgan, R. D., Posfai, J., & Raleigh, E. A. (2001). Discovery and distribution of super-integrons among Pseudomonads. *Molecular microbiology*, *42*(3), 587-601.

225. Vakulskas, C. A., Brady, K. M., & Yahr, T. L. (2009). Mechanism of transcriptional activation by Pseudomonas aeruginosa ExsA. *Journal of bacteriology*, *191*(21), 6654-6664.

226. Vallés, J., Mariscal, D., Cortés, P., Coll, P., Villagrá, A., Díaz, E., ... & Rello, J. (2004). Patterns of colonization by Pseudomonas aeruginosa in intubated patients: a 3-year prospective study of 1,607 isolates using pulsed-field gel electrophoresis with implications for prevention of ventilator-associated pneumonia. *Intensive Care Medicine*, *30*, 1768-1775.

227. Vatcheva-Dobrevska, R., Mulet, X., Ivanov, I., Zamorano, L., Dobreva, E., Velinov, T., ... & Oliver, A. (2013). Molecular epidemiology and multidrug resistance mechanisms of Pseudomonas aeruginosa isolates from Bulgarian hospitals. *Microbial Drug Resistance*, *19*(5), 355-361.

228. Veenendaal, A. K., van der Does, C., & Driessen, A. J. (2004). The protein-conducting channel SecYEG. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1694*(1-3), 81-95.

229. Vettiger, A., & Basler, M. (2016). Type VI secretion system substrates are transferred and reused among sister cells. *Cell*, *167*(1), 99-110.

230. Vidaillac, C., & Chotirmall, S. H. (2021). Pseudomonas aeruginosa in bronchiectasis: infection, inflammation, and therapies. *Expert review of respiratory medicine*, *15*(5), 649-662.

231. Visca, P., Imperi, F., & Lamont, I. L. (2007). Pyoverdine siderophores: from biogenesis to biosignificance. *Trends in microbiology*, *15*(1), 22-30.

232. Vodovar, N., Vallenet, D., Cruveiller, S., Rouy, Z., Barbe, V., Acosta, C., ... & Boccard, F. (2006). Complete genome sequence of the entomopathogenic and metabolically versatile soil bacterium Pseudomonas entomophila. *Nature biotechnology*, *24*(6), 673-679.

233. Whitney, J. C., Beck, C. M., Goo, Y. A., Russell, A. B., Harding, B. N., De Leon, J. A., ... & Mougous, J. D. (2014). Genetically distinct pathways guide effector export through the type VI secretion system. *Molecular microbiology*, *92*(3), 529-542.

234. Wilhelm, S., Gdynia, A., Tielen, P., Rosenau, F., & Jaeger, K. E. (2007). The autotransporter esterase EstA of Pseudomonas aeruginosa is required for rhamnolipid production, cell motility, and biofilm formation. *Journal of bacteriology*, *189*(18), 6695-6703.

235. Wloka, M., Rehage, H., Flemming, H. C., & Wingender, J. (2005). Structure and rheological behaviour of the extracellular polymeric substance network of mucoid Pseudomonas aeruginosa biofilms. *Biofilms*, *2*(4), 275-283.

236. Wolcott, R., Costerton, J. W., Raoult, D., & Cutler, S. J. (2013). The polymicrobial nature of biofilm infection. *Clinical Microbiology and Infection*, *19*(2), 107-112.

237. Wolfgang, M. C., Kulasekara, B. R., Liang, X., Boyd, D., Wu, K., Yang, Q., ... & Lory, S. (2003). Conservation of genome content and virulence determinants among clinical and environmental isolates of Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences*, *100*(14), 8484-8489.

238. Woods, D. E. (2004). Comparative genomic analysis of Pseudomonas aeruginosa virulence. *Trends in microbiology*, *12*(10), 437-439.

239. World Health Organization. (2017). *Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis* (No. WHO/EMP/IAU/2017.12). World Health Organization.

240. Wu, M., & Li, X. (2015). Klebsiella pneumoniae and Pseudomonas aeruginosa. In *Molecular medical microbiology* (pp. 1547-1564). Academic Press.

241. Yahr, T. L., & Wolfgang, M. C. (2006). Transcriptional regulation of the Pseudomonas aeruginosa type III secretion system. *Molecular microbiology*, *62*(3), 631-640.

242. Yan, S., & Wu, G. (2019). Can biofilm be reversed through quorum sensing in Pseudomonas aeruginosa?. *Frontiers in microbiology*, *10*, 1582.

243. Yang, L., Hu, Y., Liu, Y., Zhang, J., Ulstrup, J., & Molin, S. (2011). Distinct roles of extracellular polymeric substances in Pseudomonas aeruginosa biofilm development. *Environmental microbiology*, *13*(7), 1705-1717.

244. Yang, L., Liu, Y., Wu, H., Høiby, N., Molin, S., & Song, Z. J. (2011). Current understanding of multi-species biofilms. *International journal of oral science*, *3*(2), 74-81.

245. Zgurskaya, H. I., López, C. A., & Gnanakaran, S. (2015). Permeability barrier of Gram-negative cell envelopes and approaches to bypass it. *ACS infectious diseases*, *1*(11), 512-522.

246. Zhou, L., Wang, J., & Zhang, L. H. (2007). Modulation of bacterial Type III secretion system by a spermidine transporter dependent signaling pathway. *PLoS One*, *2*(12), e1291.

247. Zhu, M., Zhao, J., Kang, H., Kong, W., Zhao, Y., Wu, M., & Liang, H. (2016). Modulation of type III secretion system in Pseudomonas aeruginosa: involvement of the PA4857 gene product. *Frontiers in microbiology*, *7*, 7.

248. Ziha-Zarifi, I., Llanes, C., Köhler, T., Pechere, J. C., & Plesiat, P. (1999). In vivo emergence of multidrug-resistant mutants of Pseudomonas aeruginosa overexpressing the active efflux system MexA-MexB-OprM. *Antimicrobial Agents and Chemotherapy*, *43*(2), 287-291.

249. Zoued, A., Brunet, Y. R., Durand, E., Aschtgen, M. S., Logger, L., Douzi, B., ... & Cascales, E. (2014). Architecture and assembly of the Type VI secretion system. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1843*(8), 1664-1673.