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Chloramphenicol Resistance in *Pseudomonas aeruginosa*

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Abstract *- Pseudomonas aeruginosa is an opportunist bacterium that is pathogenic for immunocompromised humans. It is very difficult to control the infections caused by this organism due to the formation of antibiotic-resistant biofilms. Multidrug-resistant strains often arise due to the presence of transferable parts in the genome of P. aeruginosa. Genomic changes and other factors affect the efflux pump systems, which function for the exclusion of toxic substances from the bacterial cells and confer antibiotic resistance. Out of many efflux pumps present in this bacterium, MexEF-OprN is responsible for conferring chloramphenicol resistance to P. aeruginosa. In this review article, the role of different factors, regulators, and signals which affect chloramphenicol resistance in P. aeruginosa are discussed.*

Keywords - *Pseudomonas aeruginosa*, chloramphenicol, antibiotic resistance, efflux pump

I. INTRODUCTION

Pseudomonas aeruginosa belongs to the family *Pseudomonadaceae*. It is a bacillus and gram-negative. It is a ubiquitous organism that is a common inhabitant of soil and water [1]. It possesses a single polar flagellum due to the virtue of which it is motile. *P. aeruginosa* is recognized as an aerobic bacterium. However, it is found to survive in anaerobic conditions as well upon the availability of certain nutrients, like nitrate [1]. It is not categorized under a specific environmental temperature range, as it is quite versatile and can withstand a range of temperatures; 4 °C to 42 °C [2]. Its nutritional versatility is also evident, given that it can utilize various carbon sources.

II. STRAINS OF P. AERUGINOSA

Studies have shown the classification of *P. aeruginosa* strains into two major groups. One bigger group contains the most studied strain PAO1 and some other notable cystic fibrosis strains, while the second group contains the virulent strain PA14 and some multidrug-resistant strains of *P. aeruginosa*[3]. The strain PAO1 is generally considered a model for research purposes. PAO1 is originally a strain of Australia [4] but has been sequenced for research purposes by the University of Washington. Hence it is named PAO1-UW [5, 6]. A subline of the strain, MPAO1, has been used to maintain mutant transposon libraries of nearly every gene that has been found in the *P.aeruginosa* [7]. Throughout the world, there has been diversifying the phenotypes observed for *P. aeruginosa* within the culturing of the PAO1 strains. The major differences in the subline MPAO1 compared to PAO1 comprise a large inversion, 39 single nucleotide substitutions (SNSs), duplication of a mobile element, and several deletions [6].

III. GENOME OF P. AERUGINOSA

Pseudomonasaeruginosa contains a genome of 6.3Mbp, and it has been successfully sequenced and well-studied. The genome of *P. aeruginosa* has been characterized gene by gene and is available to the scientific community on the internet under the web address www.Pseudomonas.com. The genome has also been published in Nature [5]. There are almost 5570 characterized genes of *P. aeruginosa* genome. Out of these, almost 10% are known to have shown homology with various regulators [8]. With such a vast variety of regulators, homologous to the other genera, *P. aeruginosa* can thus survive and adapt in a variety of habitats which includes the human body. An opportunistic bacterium is a pathogen that does not cause infection unless the immunity of an individual is compromised. For instance, in patients with leukemia, cancer, HIV, wounds, burns, and other illnesses where immunity is weak, *P. aeruginosa* is pathogenic. Otherwise, the bacterium lives as a commensal as a part of normal flora.

IV. HUMAN INFECTIONS

P. aeruginosa is an opportunistic pathogen. It has a range of potential infectious areas within the human body due to its diverse habitat preferences. The infections caused by the bacterium are generally nosocomial as hospitalized patients are usually immunocompromised and hence at a greater risk for infection. Due to its versatility of growth conditions, *P.aeruginosa* can easily survive in nooks and crannies of the minimalistic hospital environment. It has also been known to develop resistance against various antibiotics, which help in its survival despite the use of antibiotics [9]. Emergence of multidrug resistant (MDR) *P.aeruginosa* is not uncommon [10,11]. The infections caused by *P. aeruginosa*

are diverse and associated with various parts of the body. The most commonly known infection is a chronic lung infection which can lead to deleterious conditions if worsened, for instance, pneumonia [12, 13]. Lung infections are seen specifically in patients with cystic fibrosis [14]. Other infections include corneal infections in individuals using contact lenses and burn wound infections [15, 16]. It is noteworthy that all these infections occur when the patient is immunocompromised. Cystic fibrosis is characterized by a genetic alteration in a transmembrane conductance regulator, also known as CFTR [17, 18]. The truncated protein produced from this gene causes unnecessary mucus secretion in pulmonary cavities [19]. This provides a habitable environment for *P. aeruginosa* to grow [20]. Contact lenses, when improperly implanted in the eyes, cause scratches over the corneal surface. This aids in the invasion of *P. aeruginosa* into the cornea and causes keratitis [21]. Burn patients of the third degree are exposed to the open environment and are at great risk for infection. Because of the loss of their skin barrier, they are left with a humid environment for *P.aeruginosa*, whether from normal skin flora or from the environment to invade the wound[22].

V. VIRULENCE FACTORS OF P. AERUGINOSA

The infections caused by *P. aeruginosa* in humans are attributed to the virulence factors produced by the pathogen. Among the numerous virulence factors, exotoxin A and alginate are potent ones [23]. Each virulence factor is specialized in its area of action. For instance, exotoxin A has eukaryotic cells as its target, whereas alginate is a survival technique to ward off-host immune responses and helps resist antibiotics [24- 27].

Virulence factors are still vague and less understood in science [28]. According to one school of thought, a virulence factor is a substance produced by a bacterium that harms the host. On the other hand, some people are inclined to define a virulence factor as a trait which aids a bacterium in survival within a susceptible host's body [29,30]. If a bacterium lacks the ability to survive in a host for long, it won't be able to be recognized as a chronic pathogen. Therefore, survival is crucial for a pathogen, and it is quite possible that the pathogenic symptoms seen in the host are merely a consequence of the survival. *P.aeruginosa* produces factors that are both virulent and crucial for survival, as mentioned earlier. Exotoxin A is known to hamper the protein synthesis in a eukaryotic cell [31]. This causes apoptosis which leads to the release of nutrients on which this pathogen survives [32].

VI. ANTIBIOTIC RESISTANCE

Gram-negative bacteria like *Pseudomonas aeruginosa* are known pathogens in hospital settings. Biofilms produced by this pathogen are resistant to most antibiotics. Moreover, a large part of the P. aeruginosa genome is comprised of transferable elements which help bacteria to develop antibiotic-resistant strains [3]. Generally, antibiotic resistance in *P. aeruginosa* is attributed to the efflux pumps that these bacteria synthesize. Efflux pumps help bacteria to throw the toxic substances out of the cell into the external environment, thus making them resistant to toxic antibiotics [33].In *Pseudomonasaeruginosa,* there are four well-characterized efflux systems; MexA-MexB-OprM, MexC-MexD-OprJ, MexE-MexF-OprN, and MexX-MexY-OprM; each pump possesses a specific preference for a substrate [34]. These pumps are expressible given various external factors like quorum sensing molecules, low pH, iron availability, etc. They are generally overexpressed in the stationary phase of growth [35].

VII. EFFLUX SYSTEMS OF P. AERUGINOSA

The major systems contributing to intrinsic and/or acquired multidrug resistance include MexAB-OprM, MexXY-OprM, MexEF-OprN, and MexCD-OprJ (Ramos, 2004). Unlike MexAB-OprM and MexXY-OprM, which contribute to intrinsic resistance [36], the MexEF-OprN and MexCD-OprJ systems are typically quiescent in wild-type cells under usual laboratory growth conditions [37].*MexEF-OprN* genes do not show any expression in the PAO1 strain owing to 8bp mutation in *mexT* gene resulting in non-functional protein to regulate mexEF-OprN expression. *mexT*, which occurs upstream of *mexEF-oprN* and downstream of *mexS*, encodes a LysR family positive regulator that promotes *mexEF-oprN* and *sex* expression. Moreover, a mutation in the *mexS* gene encoding an oxidoreductase of unknown function results in the silencing of the MexEF-OprN system[38]. *mexEF-oprN*expression and modest multidrug resistance have also been reported for a mutant disruptedin the *mvaT* gene, encoding a global regulator of virulence gene expression.

VIII. MEXEF-OPRN EFFLUX PUMP

Like other tripartite RND family pumps, the MexEF-OprN efflux system consists of an inner membrane, drug-proton antiporter (the RND component) (MexF), an outer membrane channel-forming component (OprN), and a periplasmic membrane fusion protein (MexE). Originally identified as a determinant of fluoroquinolone resistance, MexEF-OprN accommodates a variety of antimicrobials, including trimethoprim and chloramphenicol, etc. Enhanced resistance to imipenem, which is also seen for *nfxC* and *mexS* mutant strains, results not from *mexEF-oprN* expression but from the concomitant decrease in levels of the outer membrane protein OprD [37]. OprD is an imipenem channel and a primary route for the entry of this antibiotic in *P. aeruginosa*, whose absence is often seen in imipenem-resistant strains of *P. aeruginosa*.

IX. REGULATION OF MULTIDRUG RESISTANCE

Despite the identification of regulatory genes impacting the expression of *mexEF-oprN*, little is known about the signals to which the regulators respond in

promoting efflux gene expression, and in fact, it is far from clear that antimicrobial efflux and resistance are the intended functions of this pump. For example, mutants overexpressing MexEF-OprN were readily recovered from an experimental model of rat pneumonia in the absence of antibiotic selection, indicating some advantage of MexEF-OprN expression *in vivo*, independent of antimicrobial export [39]. Transcriptome analysis of *P. aeruginosa* exposed to airway epithelial cells has revealed substantial increases in the levels of expression of *mexEF-oprN* and *mexS* (PA2491), apparently in parallel with increased damage of the epithelial cells and, therefore, the release of cell contents, although the efflux-recruiting signal(s) released by these cells is unknown. The observation that a mutational loss of the VsqR quorum-sensing (QS) and virulence regulator compromises *mexEF-oprN* expression in cells under conditions of oxidative stress suggests that this system may normally be induced in response to this stressor (Figure 1). Finally, a synthetic derivative of a natural furanone compound that functions as an antagonist of QS in *P. aeruginosa* was also shown previously to induce *mexEF-oprN* expression [39].

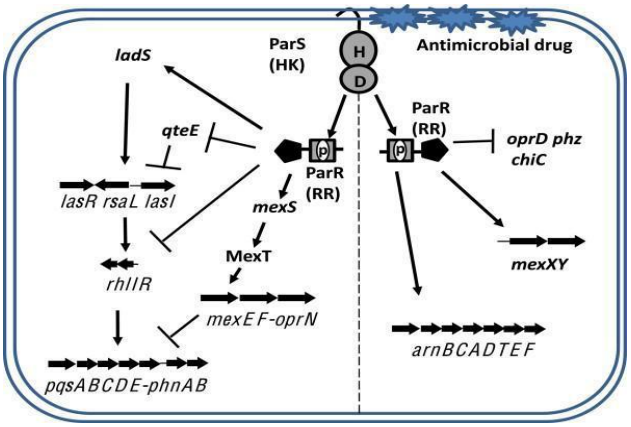

Figure 1: Genes involved in modulation of chloramphenicol resistance in P. aeruginosa [35].

X. QUORUM SENSING

Quorum sensing is a complex system of factors, including regulatory factors as well as virulent factors. Quorum sensing is a system that requires a threshold level of cells to be surpassed, beyond which the system activates fully [40]. The system benefits the cells of a bacterial population to communicate with each other. *P. aeruginosa* possesses two quorum-sensing systems, which are well characterized; *las* and *rhl* systems. Each of these systems has its own autoinducer synthase, which makes an autoinducer molecule which is an identification marker of that system. This autoinducer molecule is usually a homoserine lactone which functions by being transported out of the cell to affect other cells [16]. These systems are named so because of the cell density dependency, which, when achieved (quorum), the autoinducer molecules subsequently also reach the required level to be impactful. The extracellular environment then becomes a complex network of molecules that enter the neighboring cells and attach to transcriptional regulators [41]. The activation of a transcriptional regulator is followed by the transcription of a cascade of genes [42]. The *rhl* system has the autoinducer synthase *RhlI*, which synthesizes C4-HSL, the transcriptional regulator for which is *RhlR* [43]. The activation of *rhl* system affects downstream the synthesis of rhamnolipids, which is a hemolysin and a biosurfactant, and pyocyanin which is a bluish-green pigmented toxin [44]. The second quorum sensing system is *las* which is composed of the autoinducer synthase *LasI* the product of which is 3-oxo-C12-HSL. The transcriptional regulator here is *LasR*. The activation of the *las* system governs the features of exotoxin A, Pyoverdine, and alkaline protease [44]. It has been found that quorum sensing systems coordinate with each other as well, instead of working as independent entities [45]. This indicates the complexity of quorum sensing in bacteria. Quorum sensing is reported to regulate biofilm development, which is considered another virulence factor of *P. aeruginosa* [46,47]. Along with *N*-acyl-homoserine lactones (*las* and *rhl* systems), the quorum sensing system also contains the 4-quinolones [48, 49] (Figure 2). For biofilm development and various other stress responses, the latter plays a major role [50]. The 4-quinolones are known to be over 50. However, the most potent and well-studied is the 2-heptyl-3-hydroxy-4-quinolone, also known as the pseudomonas quinolone signal (PQS) [48]. PQSauto regulates its production via the PQS operon consisting of 4 biosynthetic genes [49]. It is packaged into membrane vesicles. PQS is regulated by *LasR* and PQS in turn, influences *RhlI* [52].

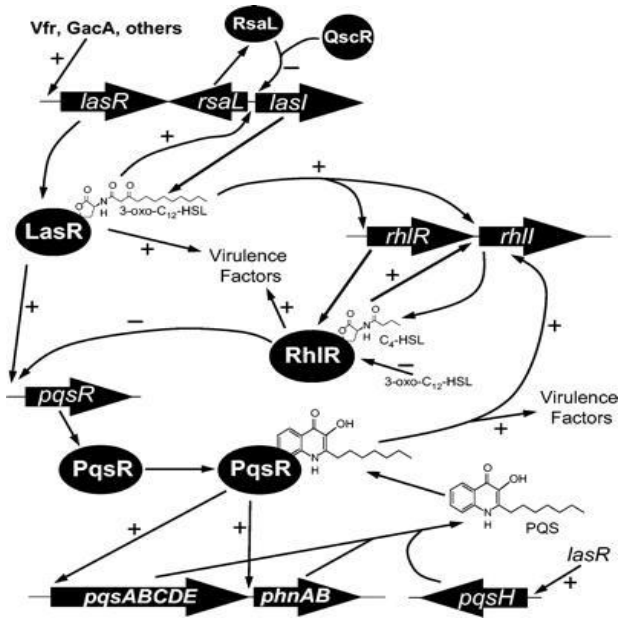

Figure 2: Hierarchy of quorum sensing system of pseudomonas aeruginosa(Adapted from [53]).

XI. ROLE OF PVC OPERON IN ANTIBIOTIC RESISTANCE

The *pvc* operon is a cluster of four genes *pvcA* (PA2254), *pvcB* (PA2255), *pvcC* (PA2256), and *pvcD* (PA2257), and is regulated by a transcriptional regulator *ptxR* laying adjacent to the *pvc* operon [54]. This operon has been linked to the production of pyoverdine chromophore and pseudoverdine [55]. Pyoverdine is a siderophore of *P.aeruginosa*, which contains a tricyclic chromophore embedded in its cyclic peptide moiety. The chromophore was thought to be formed by two potential genetic loci; *pvdL*, which is a nonribosomal peptide synthase, and the *pvc* gene cluster [56]. However, the *pvc* gene cluster was overexpressed and analyzed with LC-MS and was actually found to be responsible for the production of an isonitrile functionalized hydroxycoumarin, which is now known as paerucumarin [57]. In this gene cluster, the *pvcA* gene is an isonitrile synthase, the *pvcB* genesis an oxidizing enzyme for specific to amino acids [58], also found to be an Fe, α-ketoglutarate-dependent hydroxylase [59], the *pvcC* and *pvcD* are both a two-component flavin adenine dinucleotide-dependent monooxygenases which are specialized to oxidize phenols giving off two types of products; catechols and dihydroxy phenols [56, 57]. The *pvcA* first produced isonitrile functionalized tyrosine (IFT) using tyrosine as a substrate. Then *pvcB*, which is an oxygenase/hydroxylase, oxidizes IFT to give an intermediate unsaturated compound [60]. This intermediate compound is then again oxidized to catechol by the joint action of *pvcC* and *pvcD*. This catechol cyclizes intramolecularly and carboxylated to give isonitrile functionalized dihydroxycumarin, or trivially known as paerucumarin (‘paeru’ denoting *P. aeruginosa*) [57].

Paerucumarin was previously considered a siderophore as it chelates iron. However, another study disproved that it is a classical siderophore, given that disrupted *pvcA* and *pvcB* genes did not halt the growth of *P. aeruginosa* in iron-limited conditions [59]. Paerucumarin is also known to be involved in the biofilm development of *Pseudomonas* aeruginosa. The *Pseudomonas aeruginosa*fimbrial appendages are synthesized by the *cup* genes (*cupB* and *cupC*), which are structures crucial for biofilm development and adherence to surfaces. MPAO1 harboring mutations in the *pvcABCD* operon hampers biofilm production and expression of fimbrial biosynthetic genes [54]. The cellular localization of paerucumarin was found to be not associated with the cell membrane. Instead, it is a diffusible molecule [61]. Mutant of pvc operon (PW4832) shows susceptibility to chloramphenicol and ciprofloxacin as compared to the resistant parent (MPAO1) and modulation of MexEF-OprN pump [62].

XII. CONCLUSION

Antibiotic resistance studies in *P. aeruginosa* are of utmost importance since this pathogen is included in the WHO list of important pathogens. A study of the mechanism of resistance and the factors involved in MDR strains could help in the development of suitable antibiotics and relevant therapeutic development.

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