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Review Article

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Quorum Sensing and Some Promising Virulence Inhibitors in *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is a Gram-negative bacterium frequently found in clinical specimens of hospitalized patients, mainly those with suppressed immune systems. It is one of the topmost ubiquitous nosocomial pathogens in humans and is considered life-threatening. *Pseudomonas aeruginosa* is the chief causative agent of pneumonia, urinary tract infections, surgical site infections, burns infections and infections in immunosuppressed patients. Pyocyanin, pyoverdin, exotoxin-A, rhamnolipids, and protease enzymes are only a few of the virulence factors produced by *Pseudomonas aeruginosa* that contribute to its pathogenesis. Long-term treatment of antimicrobial agents resulted in the development of multi-drug resistant strains, which are highly complicated to be treated. Therefore, there is a great interest in discovering innovative strategies for managing MDR *Pseudomonas aeruginosa*. Quorum sensing inhibitors offer a substitutional policy to combat microbial infections. This policy depends on disarming microbial pathogens by attenuating virulence factors production regulated by quorum sensing and eliminating the pathogen's capacity to harm the host. It is anticipated that quorum sensing inhibitors could be valuable in treating infections caused by MDR *Pseudomonas aeruginosa* by acting as adjuvants or alternatives to conventional antibiotics. The strategy of finding quorum sensing inhibitors is beginning to produce promising outcomes.

1. Introduction

P. aeruginosa is a Gram-negative bacterium with a rod shape belonging to the family *Pseudomonadaceae*, which contains 191 known species with only 12 species that have clinical importance. The most frequently widespread species of *the Pseudomonadaceae* family is *P. aeruginosa,* the fatal opportunistic pathogen with high resistance ability to an enormous number of disinfectants and antibiotics [1]**.** After developing sterile culture media, *P. aeruginosa* was first characterized as a distinctive bacterial species in the last years of the 19th century. The high mortality rate due to infections caused by *P. aeruginosa* is the major cause that provoked expanding investigations into *P. aeruginosa* infections[2, 3].

2. Diseases caused by *P. aeruginosa*

P. aeruginosa is an important and very dangerous opportunistic pathogen causing many fatal infections in patients with severe medical conditions. *P. aeruginosa* is associated with nosocomial infections, mainly in immunocompromised patients. It is rated the third-most-common microorganism associated with hospital-acquired infections representing about 10-15% of nosocomial infections recorded globally [4-6]**.** *P. aeruginosa* is considered as a chief uropathogenic that causes complicated urinary tract infections (UTIs). Urinary tract infections, which make up 20 to 49% of all nosocomial infections, are often among the most common diseases among hospitalized patients. *P. aeruginosa* is the third most prevalent pathogen linked to hospital-acquired UTIs, and it is responsible for 7–10% of UTIs in the hospital environment. Additionally, this bacterium can cause potentially lethal sepsis from severe UTIs in older people or vulnerable hosts whose overall health is deteriorated or whose immunity is reduced as a result of diabetes or anticancer chemotherapy medications [7, 8]. Furthermore, *P. aeruginosa* is a primary cause of skin and soft tissue infections commonly isolated from severe burns and surgical wound infections. Burns and wound infections due to *P. aeruginosa* pose a significant challenge in terms of systemic sepsis, graft loss and prolonged hospital stay. *P. aeruginosa* colonization in burns and wounds, as well as their systemic invasion, may result in serious consequences and even death. The prevalence of *P. aeruginosa* has been reported as the most common source of burn infection globally. In addition, wound infections caused by MDR *P. aeruginosa* have also been reported with high morbidity and mortality rates worldwide due to resistance to many commonly used antibiotics, which necessitate intense monitoring in wounds and burns wards [9-11]. Moreover, acute hospital-acquired pneumonia is frequently caused by *P. aeruginosa*, although community-acquired pneumonia is less frequently caused by this organism. In some reports, *P. aeruginosa* is rated second after *Staphylococcus aureus* as a causative agent of hospital-acquired pneumonia. In addition, it was previously reported that ventilator-associated pneumonia caused by *P. aeruginosa* is associated with very high mortality, in some studies, as high as 87%. Chronic respiratory tract infections like CF are also brought on by *P. aeruginosa* which typically colonizes the paranasal sinuses after being infected and adapting to the new environment in the respiratory tract. The colonizing bacteria undergo a phenotypic transformation to a mucoid phenotype with strong antibiotic resistance through mutational stages. Patients with CF are considered as the best well-known example of chronic pseudomonal lung infections [12-15]**.** In addition, *P. aeruginosa* can also cause many other severe diseases, including bacteremia, endocarditis, osteomyelitis, otitis externa and ulcerative keratitis [16, 17].

3. Virulence factors (pathogenicity determinants) of *P. aeruginosa*

The pathogenicity of *P. aeruginosa* is correlated with the production of an array of virulence factors (figure 1), resulting in severe infections which are hard to be eradicated due to the emergence of resistance against many antibiotics. The trouble is further complicated by biofilm formation, allowing safe environmental conditions for bacterial cells against stresses such as antiseptics, disinfectants and antibiotics

[18]. *P. aeruginosa* possesses multitude of virulence factors, and its virulence has been attributed to both cell-associated components like lipopolysaccharide (LPS), alginate, flagella, and pili as well as secretory virulence factors and enzymes like proteases, elastase, pyocyanin, pyoverdin, rhamnolipids, exotoxin A, and hemolysins [12]. Moreover, like many Gram-negative bacteria, *P. aeruginosa* adversely affects eukaryotic host cells with the type-III secretion system (T3SS), a crucial virulence factor that acts in a highly regulated manner through inoculation of *P. aeruginosa* toxins and enzymes into host cells cytosol [19].

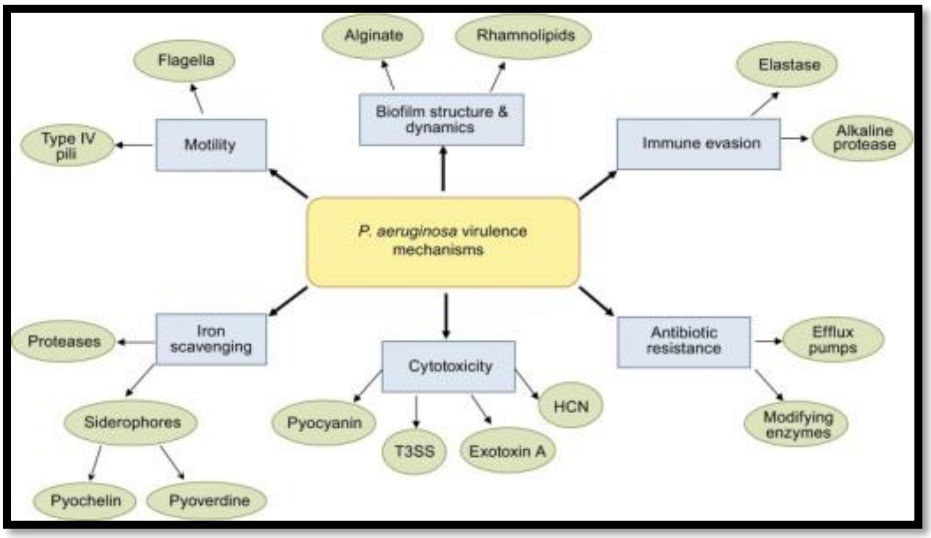
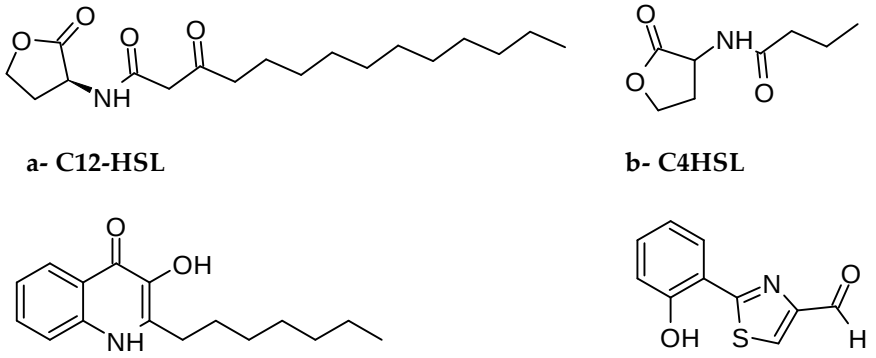


Figure (1). Mechanisms of virulence in *P. aeruginosa* infections [17].

4. Quorum sensing in *P. aeruginosa*

Understanding the pathways in *P. aeruginosa*, which regulate the expression of many virulence genes, can aid in combating bacterial infections. The research showed that bacteria assortment their behavior through a cell-to-cell interconnection mechanism known as quorum sensing (QS) that permits bacteria to regulate their virulence factors expression and production [20]. In the QS process, bacteria secrete and detect tiny signal chemical molecules, autoinducers, which function as co-factors for some transcriptional regulators. Bacteria have a specificity that allows them to identify their autoinducers. When their receptors are subjected to enough amount of a particular autoinducer, indicating that adequate bacterial cells concentration is present (quorum), they stimulate a response cascade turning on several genes associated with the regulation of different bacterial processes such as virulence factors production and biofilm formation [21-23]. QS machinery of *P. aeruginosa* has been investigated at large scale and is considered a model organism in QS research. It consists mainly of three systems, lasI/R, rhlI/R and pqsA/R, interwoven hierarchically. LasI/R system in *P. aeruginosa* is present at the top of the signaling hierarchy and is considered the chief regulator. Once stimulated, it activates both rhl and pqs systems [20, 24, 25]. LasI/R signaling system is composed of *lasI* gene, which synthesizes the autoinducer N-3-oxododecanoyl-L-homoserine lactone (C12-HSL) and the *lasR* gene, which encodes for a transcriptional activator protein. As previously reported, LasI/R regulates the production of many virulence factors such as elastase, proteases and exotoxin A [20, 26]. The second QS signaling system discovered in *P. aeruginosa* is RhlI/R. It's responsible for synthesizing another autoinducer known as N-butyryl-L-homoserine lactone (C4-HSL) encoded by the *rhlI* gene with sequence homologies *lasI*. It was reported that *rhlI* transcription is activated by lasR/C12-HSL complex and that Rhl system is Las-dependent C4-HSL doesn't interact with the LasR protein. Therefore, RhlR, another regulatory protein, was the cognate receptor of

C4-HSL. The receptor proteins LasR and RhlR are first activated and dimerized with their specified autoinducers C12-HSL and C4-HSL, respectively. The RhlR/C4-HSL complex regulates the expression of the *rhlAB* operon controls rhamnolipids and pyocyanin synthesis [27]. The third QS signaling molecule is *Pseudomonas* quinolone-based intracellular signaling (PQS), which is structurally known as 2-heptyl-3-hydroxy-4-quinolone. The first step in PQS biosynthesis is the initiation of *pqsA* gene followed by the formation of 2-heptyl-4-quinolone (HHQ), which is the precursor of PQS, converted to PQS under the control of *lasR*. This means that the PQS system is regulated by the las system [28, 29]**.** *PqsR* is the transcriptional regulator for PQS system. The expression of *pqsR* is controlled by lasR/C12-HSL. When PQS connects with its cognate receptor *pqsR*, this leads to an increase in its activity. PQS was found to play a significant role in regulating biofilm formation, secondary metabolite production, pigments and virulence factors such as pyocyanin and pyoverdin [24, 30]**.** A fourth QS system, known as the integrated quorum sensing system (IQS), has been discovered in the last few years during searching for another mechanism that may substitute las system after the appearance of clinical isolates with las system defects. IQS was structurally recognized as 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde. Disrupting the biosynthesis of IQS hinders the PQS and rhl systems. It attenuates some virulence factors such as elastase, pyocyanin and rhamnolipids, indicating the importance of this system in bacterial infection pathogen esis. The exact roles of IQS need more investigation [31]**.**



a- C12-HSL **b- C4HSL**

c- PQS **d- IQS**

Figure (2). *P. aeruginosa* QS signals structure [17].

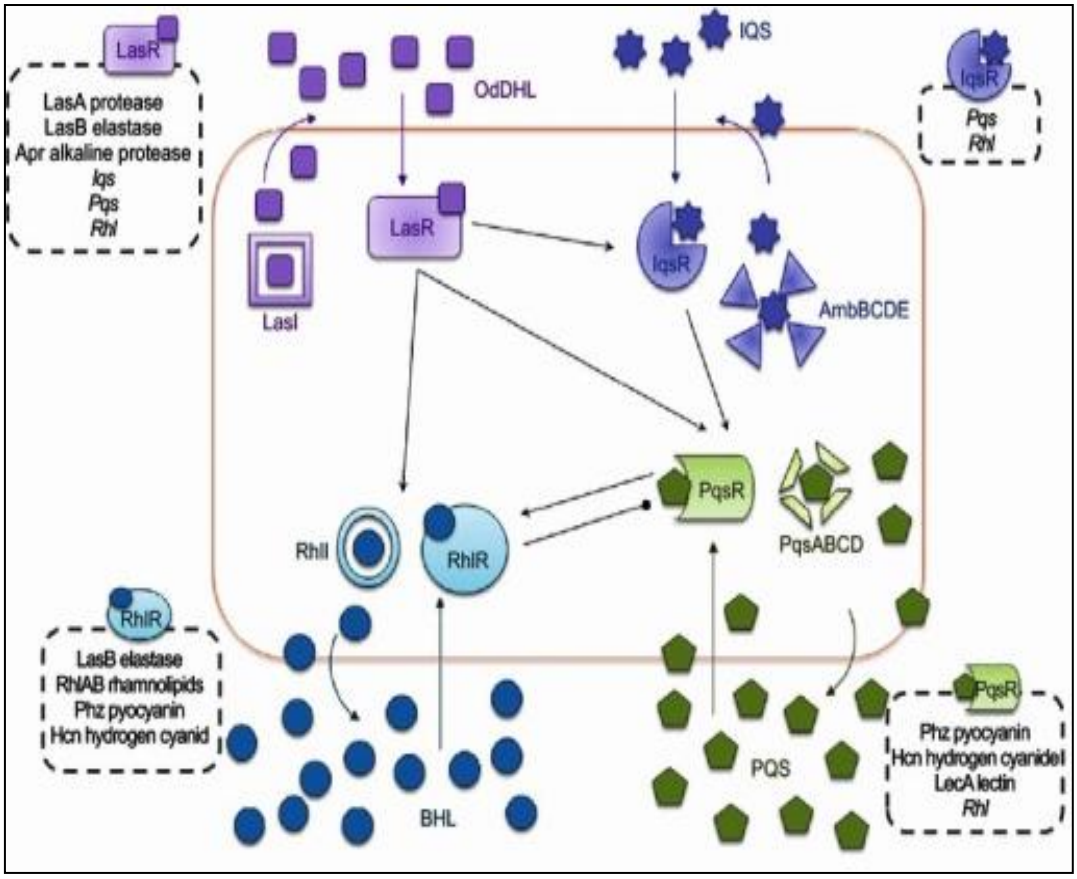


Figure (3). The four QS signaling networks in *P. aeruginosa* and their corresponding regulons [17].

5. Antimicrobial resistance in P. aeruginosa

According to the US Centers for Disease Control and Prevention (CDC), about two million US persons are infected by antimicrobial-resistant pathogens each year, resulting in about 23,000 deaths. *In addition, P. aeruginosa* infections are highly problematic to be treated due to their natural resistance to many antibiotic groups and their ability to acquire resistance against different antibiotics. Therefore, MDR *P. aeruginosa* isolates are considered as a severe problem. Nosocomial outbreaks of *P. aeruginosa* infections caused by MDR strains have become a massive threat in hospitals [32, 33]. All antibiotic resistance mechanisms were found in *P. aeruginosa*, including intrinsic, acquired and adaptive resistance and sometimes all may occur within the same isolate. Despite using antibiotic combination therapies to manage *P. aeruginosa* infections, the rate of bacterial resistance development is still increasing [34, 35]**.** *P. aeruginosa*'s intrinsic resistance is attributed to the low permeability of its outer membrane, which is potentiated with the expression of membrane efflux pump (Mex) genes. It is known that the outer membrane of *P. aeruginosa* is 10 to 100 fold less permeable than *Escherichia coli*'s outer membrane because of the presence of fewer large porins and a lot of smaller ones. Therefore, efflux pumps in *P. aeruginosa* can drive out numerous antibiotics such as β-lactams, chloramphenicol, fluoroquinolones, macrolides, novobiocin, sulfonamides, tetracycline, trimethoprim and aminoglycosides [36, 37]**.** On the other hand, acquired resistance is artificially gained through genetic transfer. Moreover, genetic mutations could also promote acquired resistance mechanisms of *P. aeruginosa*. Adaptive resistance can be induced by environmental conditions such as exposure to sub-inhibitory concentrations of some antibiotics, biofilm formation, swarming motility, and epithelial surface adherences that increase this resistance type [37]**.** In the existing battle between humans and pathogens, the balance looks to be swinging in favor of the

pathogens. Besides the slowing steps in attempts to find novel antibiotics in the past years, the rapid expansion in antibiotics resistance resulted in limited options to deal with microbial infections. The problem is, how can we ensure that the balance gets back in favor of humans? One logical reply is that we have to change the methods used to manage infections and find new treatment strategies. An unconventional approach to controlling bacterial diseases is to search for drugs that disarm pathogens without affecting bacterial growth. This idea concentrates on creating or finding drugs that inhibit bacterial virulence factors controlled by QS rather than bacterial viability [37].

6. *P. aeruginosa* quorum-sensing inhibitors

Many studies have been carried out in the last decades to find potential QS-inhibiting compounds to treat *P. aeruginosa* infections. Due to the vital link between QS and virulence factors production in *P. aeruginosa*, it is anticipated that QS inhibition could fight this bacterial pathogenicity and increase its sensitivity against antibiotics and the host immune system [38, 39]. The disruption of QS can be accomplished by interfering with acyl-homoserine lactone (AHL) signaling production, preventing the accumulation of AHL signals after synthesis and secretion by destruction or inactivation and prohibiting signal reception by blocking the binding of the signal to the receptor or by damaging the receptor protein [40]. Despite the fact that several chemically synthesized substances exhibited strong QS inhibitory action, the majority of these substances are cytotoxic, which prevents their usage in humans. Many FDA-approved medications are being examined for their potential to have QS inhibitory effects in an attempt to deal with this issue [26].

6.1. Non-steroidal anti-inflammatory drugs

Tenoxicam is a common analgesic and anti-inflammatory drug that belongs to the non-steroidal anti-inflammatory drug class. Tenoxicam was observed to considerably speed up the healing of skin wounds with *E. coli* infection in vitro in combination with antibiotics, regardless of the bacteria resistance [41-44]. In a previous study, tenoxicam was reported to have QS inhibitory activity in *P. aeruginosa* both phenotypically and at the molecular level. In addition, many other non-steroidal anti-inflammatory drugs such as aspirin and diclofenac sodium showed a potent QS inhibitory effect on *P. aeruginosa* leading to inhibition of QS-dependent virulence factors including, elastase, total protease and pyocyanin without affecting bacterial growth [26, 44, 45].

6.2. Secnidazole

Secnidazole effectively treats many infections caused by anaerobic pathogens and its role in controlling amoebiasis, giardiasis, and bacterial vaginitis. Secnidazole has structural similarities with 5-nitroimidazoles, metronidazole, a drug that has been shown to decrease the development of virulence factors by the pathogen *P. aeruginosa* by acting as an analogue of acyl-homoserine lactones (signaling molecules). Also, it was proved that secnidazole showed a potent QS inhibitory activity against different virulence genes regulated by QS [46].

6.3. ZnO nanoparticles

The use of nanoparticles in medicine and as a treatment for infectious diseases has grown recently as a result of nanotechnology advancements. The FDA has approved ZnO nanoparticles for the treatment of local infections since they have been shown to have high antibacterial action and considerably decrease skin infections and inflammation. ZnO nanoparticles have the ability to damage the integrity of bacterial cell membranes, decrease the hydrophobicity of cell surfaces, and suppress the transcription of oxidative stress-resistance genes in bacteria, all of which can cause bacterial degeneration and ultimately bacterial cell death. Moreover, ZnO nanoparticles effectively exhibited a QS inhibitory activity, as previously proved [46-48].

In addition, it was previously reported that some metal nanoparticles, such as silver showed anti-virulence activity through disruption of QS circuits with subsequent inhibition of virulence factors production [47].

6.4. Proton pump inhibitors

Pantoprazole is a member of the proton pump inhibitors group, which is routinely used in clinical practice as an adjunct to antimicrobials in treating peptic ulcers of microbial origin. Besides their inhibitory effect on acid secretion, proton pump inhibitors have also been shown to exert an antibacterial activity *in vitro*, selective to *Helicobacter pylori*. Some proton pump inhibitors do not exhibit antimicrobial activity when used alone but are reported to have a direct effect on the proton pump of certain bacterial species such as *Streptococcus mutans* when used in combination with antibiotics. Previous studies have shown that the association of a proton pump inhibitor, omeprazole, with calcium hydroxide displayed selective antimicrobial activity against endodontic microbes. Moreover, it was previously reported that the proton pump inhibitor, esomeprazole, significantly inhibited biofilm formation, which is under control of QS, in biofilm-producing *Staphylococcus aureus* and *P. aeruginosa* [47, 49, 50].

6.5. Miscellaneous QS inhibitors

Many other substances have been investigated and reported to have QS inhibitory activity. For example, a lot of organisms can secrete quorum quenching (QQ) enzymes, such as *Streptomyces*, *Bacillus* and *Ralstonia* species. These enzymes hydrolyze the lactone ring or cleave the acyl side chain of AHL [51, 52]. In addition, many plants and fungal extracts were proved to act as QS inhibitors in *P. aeruginosa* due to the similarity between their chemical structures and AHL in addition to their signaling receptor degradation, such as ajoene in garlic extract, eugenol in clove extract, ellagic acid derivatives in *Terminalia chebula*, extract of *Terminalia bellerica* and penicillic acid and patulin from *Penicillium* species [53, 54]**.** Moreover, many previously reported compounds showed QS inhibitory effect depending on inhibiting receptor-autoinducers binding. This is due to structural similarity with autoinducers such as, natural halogenated furanones produced by macroalgae, *Deliseu pulchra*, 4-nitropyridine-N-oxide, synthetic S-phenyl-L-cysteine sulfoxide and diphenyl disulfide organosulfur compounds [55, 56]**.**

7. Conclusion

QS controls the production of many of virulence factors in *P. aeruginosa* and plays an essential role in antimicrobial resistance. The rampant overuse of antibiotics increased the problem of MDR *P. aeruginosa* to many of the antibiotics available, leading to the limitation of treatment options in many infectious diseases, so new approaches to combat MDR *P. aeruginosa* have become a must. QS inhibitory agents are considered a promising pathway to deal with MDR *P. aeruginosa*, which needs more effort and effective investigations to control such a problem.

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