A review on pathogenesis associated with *Pseudomonas aeruginosa* and its modulation through paerucumarin

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Abstract
*Pseudomonas aeruginosa* is a formidable environmental bacterium and a list of nosocomial infections is attributable to it. *P. aeruginosa* is emerging as a primary cause of mortality among patients with immunodeficiency, cystic fibrosis and burn wounds worldwide. *P. aeruginosa* can switch from unicellular planktonic form to multicellular biofilm form to acquire a shield against host-immune response and antibiotics that supports its persistence in diverse niches. Moreover, inherent resistance to multiple groups of antibiotics, coupled with its acclaimed ability to acquire resistance against any antibiotic, further complicates the therapeutic options. *P. aeruginosa* acts as a linchpin to study drug resistance, quorum sensing, and quorum-based biofilm formation amid gram-negative bacteria. This review article encompasses various aspects of *P. aeruginosa*’s infection profiles and genetic determinants responsible for its virulence. By shedding light on intricate interplay between novel metabolites such as paerucumarin and manifestation of infection by *P. aeruginosa*, this publication is instrumental to recognize emerging therapeutic targets and improving treatment modalities.

Keywords: Biofilm formation; Paerucumarin; *Pseudomonas aeruginosa*; Quorum sensing; virulence

Introduction
*Pseudomonas aeruginosa* is virulent gram-negative bacilliform bacterium. It is classified under the bacterial family *Pseudomonadaceae* [1]. Carle Gessard in 1882, while studying blue and green coloration of bandages provided the earliest description of *P. aeruginosa* [2]. It is a pathogenic bacterium. *P. aeruginosa* is a prominent gram-negative species among bacterial pathogens which is ubiquitously found in the environment [3]. Targets of its virulence not only include nematodes, plants, amoeba and insects but also mammals [4-7]. It is particularly notorious for causing serious illnesses in human beings. The organism opportunistically causes nosocomial infections [8]. It is notably the underlying cause of chronic respiratory tract infections among patients with cystic fibrosis [9, 10]. The range of acute and chronic infections caused by *P. aeruginosa* are owed to its metabolic diversity and the abundance of virulence factors. Constrained permeability of outer membrane and multitude of efflux pumps grant it with constitutive resistance to
myriad antibiotics. Furthermore, rapid development of antibiotic resistance is supported by chromosomal mutation or acquisition of genes through horizontal transfer. Due to rising antibacterial resistance and bacterial virulence, *P. aeruginosa* has been categorized by World Health Organization as a “priority pathogen”, necessitating urgent development of new antibiotics against it (World Health Organization, 2017).

**Ecological significance**

It is omnipresent in all animal/human-impacted environments including water and soil [11]. It exhibits commendable flexibility in respiratory metabolism and possesses extensively branched electron transport chain with multiple options for terminal oxidases, denitrifying enzymes and oxidants that support its persistence in aerobic as well as anaerobic settings [12]. *P. aeruginosa* exhibits dissimilatory nitrate reduction, utilizing nitrogen oxide as alternate electron acceptor in electron transport chain [13]. This biological process is defined as denitrification and holds ecological significance because it represents one of the few mechanisms to generate atmospheric N₂. In anaerobic settings, arginine serves as energy source for *P. aeruginosa*. Another ubiquity determinant feature of *P. aeruginosa* is its ability to withstand a great temperature ranges from 4 ℃ to 42 ℃ while being mesophile bloom at 37 ℃. *P. aeruginosa* is a chemoheterotrophic bacterium having impressive capacity to utilize diverse array of organic compounds as energy substrate [14]. It is a key soil bacterium playing a crucial role in breakdown of polycyclic aromatic hydrocarbons.

**Genomic composition**

The complete genome of *P. aeruginosa* was first sequenced by Stover and his colleagues in 2000 [11]. It spans 6.3 Mbp in size which is a remarkably large genome comparing to *Escherichia coli* with a 4.64 Mbp genome length, *Staphylococcus aureus* with 2.81 Mbp genome and *Haemophilus influenza* with 1.83 Mbp genome length [15]. The genome of *P. aeruginosa* contains higher proportion of the regulatory genes compared to other sequenced species. The genetic sequence of *P. aeruginosa* has been documented and is accessible at [www.pseudomonas.com](http://www.pseudomonas.com), an extensively curated web-based genome resource. In addition, expertly curated metabolome database of *P. aeruginosa* is PAMDB ([http://pseudomonas.umaryland.edu/](http://pseudomonas.umaryland.edu/)). *P. aeruginosa* genome contains relatively higher GC content (66.6%), 89% of coding region and 5570 functional genes. Functional annotation of 32% of the genes is still pending.

**Human infections**

Humans encounter *P. aeruginosa* commonly in various settings and can ingest it through water and food but it does not appreciably colonize within the healthy human host [16]. Instead, it requires specific conditions to cause infection, including tissue damage and specific host-immune defects. *P. aeruginosa* is categorized as opportunistic bacterium as it predominantly infect severely ill or immunodeficient patients [17, 18]. It has emerged as notable source of healthcare infections, including intensive care unit infections, ventilator acquired pneumonia, postsurgical infections, urogenital infections, keratitis and otitis media [19, 20]. Whereas high mortality rate has been specifically observed in cases where patients are diagnosed with neonatal infections, cystic fibrosis, burn wound infections and cancers [20, 21]. The *P. aeruginosa* related infections can also pose life threatening risks when inadequate therapy is applied, especially while dealing with its multidrug-resistance variants. Multidrug resistance has been a concern for animal and human health for last three decades. Besides, *P. aeruginosa* ranks among
the most prevalent pathogens found in the hospital settings, contributing to over 50% healthcare-associated infections. Despite the development of new antimicrobial drugs, mortality rate attributed to *P. aeruginosa* is still high, ranging from 20 to 60% [19]. The primary risk elements for the infections of *P. aeruginosa* are skin burns, hematological neoplasm, structural lung diseases, recent usage of antibiotics, transplantations, presence of implants, mechanical ventilation and prolonged hospitalization [22]. It is grouped within the deadly ESKAPE bugs which represents *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter* species.

**Virulence factors**

The virulence of a pathogen is characterized by its capacity to infect the host and provoke clinical symptoms by utilizing various factors that aid in the bacterial attachment, colonization and invasion. This also involves disruption of host tissue cohesion, defense from host immunity and depletion of host nutrients. *P. aeruginosa* is equipped with an array of cellular and extracellular virulence factors like secretion systems, toxins, proteases, Lipopolysaccharides (LPS), Pilii, flagella, quorum sensing and quorum-based biofilm. Every virulence factor operates uniquely, with its designated realm. Flagella and Type IV Pilii significantly contributes to bacterial attachment to host cells, motility and biofilm formation. The secretion systems that includes Type I secretion system (T1SS) to T6SS, enables *P. aeruginosa* to inject toxins and effectors efficiently into the host cells [23]. Moreover, Exotoxin A, Exolysin, Lipase A, Phospholipase A, Pyocyanin, Leukocidin and Lipoxygenase impairs host cell functions, suppress immune response and induce cell death [23]. These virulence factors collectively contribute to pathogenicity of the *P. aeruginosa* and its ability to cause infections with severe consequences.

**Biofilm development**

One of the prominent virulence traits exhibited by *P. aeruginosa* is formation of biofilm which are stationary and well organized bacterial communities enclosed by a protective matrix of polymeric substances like exopolysaccharides (EPS), proteins and DNAases, gripped onto a substrate or to one another and displayed an atypical phenotype in term of the growth rate and gene expression [24]. This adaptation allows the bacteria to cope with environments where planktonic growth may be compromised. Biofilm development is a common feature in different *P. aeruginosa* infections which includes otitis media, endocarditis and chronic pneumonia associated with cystic fibrosis [25]. Medical devices such as contact lenses, central neural catheter and implant urological catheter can serve as platform for biofilm development by *P. aeruginosa* [26]. The establishment of biofilm involves several phases. Initially, bacteria loosely stick to conditioned surface, leading to irreversible adherence, growth and biofilm maturation. As maturation progress, bacterial multiplication occurs which leads to micro-colonies establishment and the generation of an EPS matrix encasing them. At last, bacterial focal dissolution take place from the matrix to colonize alternate surfaces [27].

**Quorum sensing system**

It is an intracellular communication mechanism that requires a variety of diffusible molecules (auto-inducers) discharged into vicinity to facilitate unified bacterial reactions during their community-based growth [28]. The Quorum sensing system of *P. aeruginosa* has been categorized into four types; pqs (Pseudomonas quinolone signal), rhl (rhamnolipids), las (LasI-LasR) and the newly integrated quorum sensing (IQS). The functioning of IQS in *P. aeruginosa* remains unexplored [29]. The las
system is centered around LasI synthase, that triggers the production of N-3(oxododecanoyl)-L-homoserine lactone. It is a signaling molecule recognized by LasR (transcriptional regulator) which activate expression of target regulon. The rhl system involves RhlI synthase producing N-butanoyl-L-homoserine lactone (C4-HSL) which is recognized by RhlR and activate expression of target regulon [30]. LasR takes on an early activation role and oversees the RhlR’s expression [31]. Both systems are pivotal in regulating the expression of genes linked to virulence factors and biofilm establishment [32]. According to Gilbert et al. [33] LasR regulates psl (P. aeruginosa exopolysaccharide) gene expression by interacting promoter region of psl operon. On the other hand, rhl system takes part in biofilm development by regulating pel polysaccharide production [34]. The P. aeruginosa’s pqs system produces 2-heptyl-3-hydroxy-4-quinolone (PQS) to signal PqsR which mediates extracellular-DNA discharge in biofilm development [35]. Moreover, it also modulates a variety of metabolic pathways such as rhamnolipid and elastase secretion, and is also linked to virulence, notably in cystic fibrosis patients [36]. Within the IQS system, 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (HHQ) is generated as autoinducer and subjects to regulation under PhoB (phosphorylated-dual component regulatory unit) and las. IQS coordinates the biosynthesis of C4-HSL, PQS and key virulence factors including elastase, pyocyanin and rhamnolipids [36]. Quorum sensing is indirectly associated with biofilm formation since it regulates twitching motility along with swarming motility, lectin and rhamnolipid biosynthesis [34]. Swarming motility is a regulated surface displacement which holds significance in initial phases of biofilm development [37]. While twitching motility is a mode of translocation which does not require any flagella and is helpful in micro-colony formation [38]. Rhamnolipid supports biofilms by maintaining open channel, forming micro-colonies, facilitating mushroom like spatial structure and promoting cell dispersion [39]. The LecA and LecB are polysaccharophilic soluble lectins which also takes part in biofilm development [40].

pvcABCD operon
The pvcABCD operon in P. aeruginosa comprises of four genes, namely pvcA, pvcB, pvcC and pvcD [41]. The expression of PvcA-D is upregulated by ptxR which is present upstream of pvc operon. The pvc genes collectively produce paerucumarin in a sequential metabolic reaction. PvcA is an isonitrile synthase which utilizes tyrosine and generate isonitrile functionalized tyrosine (IFT). PvcB is an iron-α-ketoglutarate dependent oxygenase [42] which catalyzes oxidation of IFT and produces an intermediate unsaturated compound. The PvcC and PvcD are dual component monooxygenases which requires flavin-adenine-dinucleotide as coenzyme. Their primary function is to convert phenol into two distinct products dihydroxy phenols and catechol through oxidation [41, 43]. The joint action of pvcC and pvcD convert intermediate compound to paerucumarin. Paerucumarin is regarded as siderophore due it immobilize iron through chelation [44]. Never the less, a different investigation refuted its classification as a conventional siderophore, in light of the fact that mutated pvcA and pvcB genes did not arrest P. aeruginosa growth in iron scarce settings as it does not release iron once it goes back to cell. Additionally Qaisar et al. [45] demonstrated that paerucumarin upregulates iron controlled genes like pvdS but does not function as a siderophore in P. aeruginosa lacking the ability to produce siderophores; pyoverdine and pyochelin. Paerucumarin is a
recognized contributor to modulation of fimbriae chaperon/Usher pathway (cup) genes notably cupB and cupC and, consequently, the biofilm development in Pseudomonas aeruginosa [46]. The Fimbrial appendages synthesized by cup genes are center structures crucial for surfaces adherence and biofilm development in P. aeruginosa [46]. The pvcABCD operon also regulate swarming motility in P. aeruginosa [49].

Conclusion

Pseudomonas aeruginosa is a notorious pathogenic bacterium that causes many deadly infections in humans. These diseases are caused by sensing the surrounding environment, communicating with the bacterial community and the production of internal and external virulence factors. This review article encompasses the various aspects of bacterial virulence and pathogenicity. This study can help in understanding the molecular and metabolic dynamics of P. aeruginosa in various environments and can help in combating its pathogenicity. A proactive approach is required to devise anti-virulence tactics that center on quorum sensing. Further investigation is required to study paeurucumarin to get more practical methods to specifically eliminate bacteria without posing harm to mammalian cells. Additionally, there is a crucial need to investigate how biofilm growth influences tissue responses, rather than solely concentrating on treatment effectiveness in eradicating virulence-associated factors.

Authors’ contributions

Collected the data: Z Rani & A Intisar, Analyzed the data and wrote the article: Z Rani & U Qaisar.

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