American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Virulence Factors and Antimicrobial Resistance in *Pseudomonas Aeruginosa*: A Review

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Received: 2025, 15, Jun
Accepted: 2025, 21, Jul
Published: 2025, 21, Aug

Abstract: Pseudomonas aeruginosa is a human microbe that infects individuals who have chronic pulmonary obstructive, cystic fibrosis (CF), wounds, burns, carcinoma, immunodeficiency, and serious infections that require ventilation, like covid-19. These bacteria are distinguished by types of resistance mechanisms, as well as significant virulence factors such as lipopolysaccharide, alginate, and phagocytosis-resistant capsules. These bacteria can also form biofilms, which are crucial in chronic infections because they are resistant to phagocytosis and elimination by the humoral and cellular immune systems. The fast development of P. aeruginosa resistance to almost all antibiotics makes this bacterium a major health interest; hence, it is crucial to investigate the virulence factors carried by bacteria and the antimicrobial mechanisms to identify appropriate solutions to lower or limit this resistance.

1. Introduction

Rod-shaped opportunistic Gram negative (GN) *P. aeruginosa* is a member of the Enterobacteriaceae family. Plant and animal immunocompetent or immunocompromised can also be infected. With nitrate serving as the terminal electron acceptor [1], it possesses facultative respiration developed by both aerobic and anaerobic respiration. Modern medicine finds it difficult to treat this problematic microbe because of its amazing adaptability, inclination to infect immunodeficiency hosts, drug-resistance, and broad range of adaptive defences [2]. Mostly affecting intensive care units, it is a primary reason of healthcare-associated infections. Its infections, linked with a great morbidity rate, affect several groups, including those with CF infection, COPD, or healthcare-associated pneumonia [3,4]. *P. aeruginosa* possesses a lot of pathogenicity factors, involved biofilm, LPS, elastases, pigments (pyocyanin and pyoverdine), proteases, lipases, and various toxins, including exotoxin A [5]. Every factor is regulated by a set of genes; each gene might help create several virulence elements. Treatment of this bacterium can be challenging because of its natural and acquired drug resistance. It has an amazing capacity to pick medication genes resistance, migrate from one patient to another, and remain in a medical environment [6].

2. *P. aeruginosa* factors associated with virulence

Virulence is derived from the Latin virulent. Pathogenic bacteria must avoid defensive systems, proliferate, establish, and cause damage. All of that is achieved by expressing virulence factors of bacteria, which either allow bacteria adhesion, invasion, or both, and the harmfulness of pathogenic bacteria [7]. Different virulence factors are formed by the *P. aeruginosa* bacteria, including capsular polysaccharide, lipopolysaccharide (LPS), iron siderophore, fimbriae (type 1 and type 3), and toxins, as depicted in Figure (1), making it highly virulent in addition to becoming antibiotic-resistant [8].

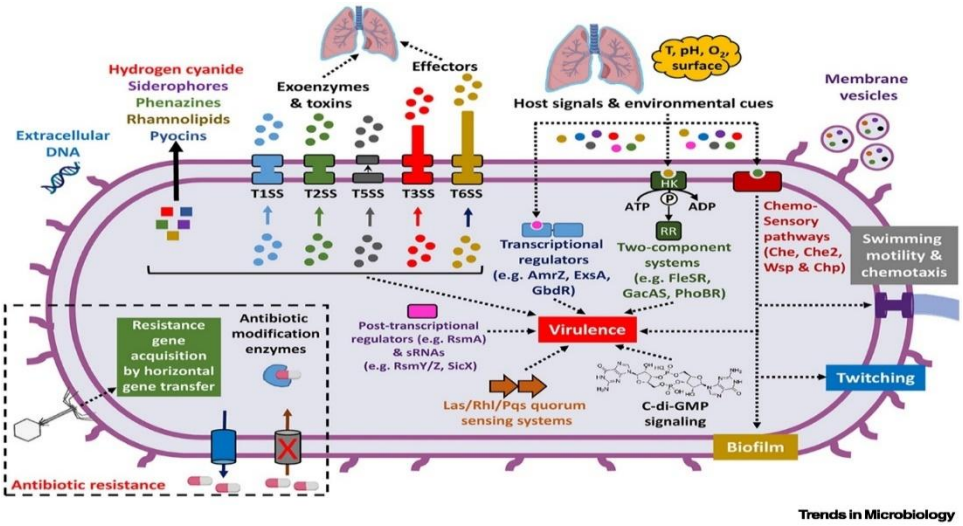

Figure 1: The most significant virulence factors that *P. aeruginosa* uses while infecting individuals (Krell and Matilla, 2024)

A. Biofilm

Biofilm is a complicated matrix created from three exo-polysaccharides (involving Pel, Psl, and alginate), polysaccharides, extracellular DNA (e-DNA), proteins, and lipids. It serves as a scaffold covering bacteria on surfaces, suppresses phagocytosis, shields them from environmental challenges like temperature variations and nutrition availability, and fosters colonization and stability [9, 10]. Well-known biofilm producer *P. aeruginosa* makes useful example for study biofilm development [11, 12]. In the polymicrobial environment of the CF lung, *P. aeruginosa* is unable to thrive, compete, or dominate without a robust biofilm [9]. *P. aeruginosa* aggressively colonizes a diverse surfaces, such as medical instruments, comprising implants, urethral catheters,

and contact lenses, as well as food processing equipment [11]. In nature, bacteria that produce biofilm can evade immune responses of the host and tolerate antimicrobial medications up to one thousand times better than their planktonic cells [13].

B. Toxins

Pseudomonas aeruginosa produces many toxins, such as four categories from type III toxins: 1) Exoenzyme Exotoxin-S (ExoS): is a secretive enzyme that functions as an ADP-ribosylase. 2) Exotoxin-U (ExoU): is a type of three-secretion mechanism for cytotoxin. ExoU interacts with phosphatase A2 and even prevents phagocyte recruitment. 3) Exotoxin-T (ExoT): is a bifunctional cytotoxin that causes death in the mitochondria of the host cells, assists in immunological escape, and interferes with the actin cytoskeleton. 4) Exotoxin-Y (Exo Y), which contains adenylate cyclase, affects the actin cytoskeleton and improves guanosine 3',5'-cyclic monophosphate (cGMP) and 3',5'-cyclic uridine monophosphate (cUMP) synthesis in host [14].

C. Lipopolysaccharide (LPS)

LPS, which include oligosaccharide- core, O antigen, and lipid A, the primary element of the OM of GN bacteria, are polysaccharide chains that extend into the external environment. Due to its position on the cell wall of bacteria, LPS is a significant chemical that encourages the host to develop an immunological defense against infections. However, LPS also helps bacteria survive in a host cells by coating them from these dangers [15]. The primary LPS virulence factor, *P. aeruginosa* GN bacteria, also expresses a key component of the characteristic cell membrane of most GN bacteria. In GN bacteria, a periplasmic medium containing a thin peptidoglycan layer separates two membranes. Lipid A, oligosaccharide-core, and O-antigen, also known as O-polysaccharide, are the three domains that makeup LPS [16].

D. Alginate

P. aeruginosa's alginate, Psl, and Pel exopolysaccharides induce a mucoid form. These exopolysaccharides suppress phagocytosis and other host antibacterial defense systems. Exopolysaccharides Psl include a lot of mannose and galactose. Psl promotes the formation of microcolonies and aids bacteria in adhering to surfaces. The exopolysaccharide pel, often known as alginate, is the major component of *P. aeruginosa* biofilms. The most studied exopolysaccharide (EPS) is made up of 1-4 connected β-d-mannuronic and α-l-guluronic acids, which are partly acetylated [17]. Even though biofilms are unnecessary, mucoid P. aeruginosa strains create an overabundance of this exopolysaccharide. It enhances its growth, structure, and stability [18, 19].

E. Enzymes

In living cells, enzymes function as biocatalysts and are including in a variety of biological reactions and cellular metabolic interactions. All distinct enzymes are found in proteins [20]. Enzymes are widely utilized in industrial, pharmacological, therapeutic, and sanitary activities because they are excellent biocatalysts for a wide range of chemical reactions. According to Meurig and Raja [21], enzymes are becoming more and more common in ecological science and environmental advancements. When infected *P. aeruginosa* generates proteolytic enzymes for instance, alkaline protease, protease IV, and, elastase which can break down collagen and elastin and cause extreme tissue damage [22]. Iron-binding proteins like lactoferrin in mucosal secretions and transferrin in serum cause ordinary conditions to restrict the normal availability of iron in the host system. A part of natural immunity, these proteins defend against pathogens. *P. aeruginosa* mostly produces pyoverdine and pyochelin, two siderophore components meant to avoid host defenses. Apart from displaying unique functional and structural features, these siderophores can chelate Fe^{+3} ions [23].

F. Secretion system (type VI)

Research on T6SS in *P. aeruginosa* has focused mostly on assembly, structure, secretion control,

and mechanical function [24]. The strain of *P. aeruginosa* that has T6SS system provides protection against other environmental bacteria and helps in eukaryotic communication. Present in *P. aeruginosa*, three type six secretion system genes: H1, H2, and H3, all have bearing on the interactions of the bacteria with other living entities. The H1seacreation system releases no less than six toxic effectors into host cells enable. This system will act as a model for examining the physiological role of T6SS antibacterial activity [25, 26]. Previous studies have indicated that *P. aeruginosa* internalisation is dependent on eukaryotic phosphoinositide-3-kinase (PI3K) activation, which results in phosphorylation of AKT in the presence of phosphatidic acid, actin modification, and extension development [27, 28, 29].

G. Pigments

Phenazines are the most prevalent extracellular pigments generated by the *Pseudomonas* genus. *P. aeruginosa* generates a water-soluble green-blue phenazine molecule known as pyocyanin pigment. Pyocyanin has a redox capability similar to menaquinone, and it has long been employed as a reversible dye. Pyocyanin has many therapeutically impacts on bacterial cells, and its physiological action is linked to chemical analogies with flavin complex, flavoproteins, nucleotides, isoalloxazine, and FAD substances [30]. The capacity of the phenazine-derived pyocyanin pigment to produce reactive oxygen species (ROS) is particularly noteworthy. Pyocyanin-generated reactive oxygen species are toxic to tumour cells because it inhibits type I and II DNA-topoisomerase activity in cells of eukaryotes [31]. By means of NADH or NADPH, pyocyanin can initiate a nonenzymatic redox cascade that inhibits microorganium proliferation. Several research suggest that pyocianin inhibits the development of fungus in living entities including humans. Additionally well-known for its bactericidal activity against other bacteria, pyocyanin lowers competition for survival and resources. Being a virulence factor, it drives the cell to lyse in the host since its solubility allows it to cross cell membranes rapidly. It also generates other colors: Pyorubrin (red), Pyomelanin (brown), and Pyoverdin (yellow; also known as pseudobactin); (greenish-yellow in color; sometimes known as pseudobactin) [32].

H. Quorum signaling (QS)

It is a technique for communication between bacterial cells through the diffusion of chemical substances. The presence of the quorum is necessary for the production of a secreted signal substance (also termed to as an auto-inducer) in sufficient quantities, which is a significant regularity. Autoinducers aggregate in the environment as the bacterial community's population increases. Both Gram positive (GP) and GN organisms use quorum signaling [33]. Three key quorum signaling systems, including Rhl, PQS, and Las systems, are responsible for coordinating the virulence genes. Toxins and proteases are the two most important pathogenic agents [34]. Pyocyanin, rhamnolipids, elastase, and alkaline protease activity are all regulated by a quorum-signaling system [35, 36].

4. The antibacterial resistance of *Pseudomonas aeruginosa*

Antibiotic resistance is the capacity of bacteria to proliferate using current antibiotics that would usually kill or prevent them. People, animals, and the environment are now seriously threatened by it [37]. Common bacteria, *P. aeruginosa* can quickly develop resistance to many wide-ranging medications. Because of its extensive distribution, clever mechanisms for antibiotic resistance, and nosocomial infections—that all point to P. aeruginosa as a multidrug-resistant pathogen—MDR prototype was found. *P. aeruginosa* has many antimicrobial agent classes, divided according to their antibiotic resistance into four groups: MDR *P. aeruginosa* is resistant to at least three different antimicrobials. P. aeruginosa has substantial drug resistance (XDR), meaning it can withstand several antimicrobial agents across all classes except two or fewer. Bacteria exhibiting the Pandemic drug resistance (PDR) phenotype are resistant to all antibacterial drugs in all classes [38]. MDR *P. aeruginosa* causes 4-60% of hospital infections, which is a growing cause of patient death and morbidity [39].

Table 1: A comparison of *P. aeruginosa* antibiotic resistance mechanisms that are inherent, acquired, and adaptive

Resistance strategy	Definition	Mechanisms
Intrinsic resistance	**Natural resistance that *P. aeruginosa* possess due to genetic and structural features**	**Decrease outer membrane permeability, antibiotic inactivation enzymes, constitutive efflux-pumps expression**
Acquired resistance	**Resistance that arise due to genetic mutation or horizontal gene transfer from other bacteria**	**Mutation (in antibiotic target or porin), transfer of resistance genes via plasmid, and bacteriophage, transposon, integron**
Adaptive resistance	**Is a temporary and reversible ability of *P. aeruginosa* to survive antibiotic exposure by control to their gene expression and physiological state**	**Biofilm formation, and quorum signaling**

4.1 Intrinsic or inherent resistance

Inherent drug resistance is the capability of microbial cells to limit the efficiency of a particular antibiotic due to inherent structural features [40]. It includes decrease outer membrane permeability, the production of antibiotic-inactivating enzymes (like ß-lactamases), and efflux pump mechanisms [41].

OM permeability

Bacteria develop resistance to hydrophilic antibiotics as a result of reduced OM permeability, which prevents the drug from reaching its target [42]. In order for the most of medications utilized to treat *P. aeruginosa* infections to reach their intracellular targets, the cell membrane must be permeable [43]. GN bacteria, including *P. aeruginosa*, possess a protective OM that functions as a semi-permeable barrier, therefore hindering antibiotic diffusion. This OM is a non-analogous phospholipid double layer and LPS [44]. Porins spread inside this structure create protein multi-meric channels. LPS itself came from O-antigen, oligosaccharide core, and lipid A. Especially organic compounds like lactic acid and citric help as chelating agents; they attach to Mg2+ cations and disturb the oligosaccharide core in the LPS substance. This association modifies the permeability of the bacterial cell wall and introduces additional layer of resistance. *P. aeruginosa* uses several porins to regulate permeability. OprB, D, E, O, and P are particular porins, whereas OprF is the primary non-specific porin. The efflux porins ask for OprJ, OprM, and OprN. Considered gated porins, OprH and OprC are OstrH and OstrC respectively [45]. Understanding these complex networks allows one to appreciate the numerous strategies *P. aeruginosa* employs to combat antibiotic penetration.

Efflux pump

Efflux pumps are transporter molecules that actively carry antibiotics among other substrates from cells to the surroundings. Additionally observed in eukaryotic cells are GP and GN bacteria [46]. Bacterial efflux pump systems are classified into five classes: ATP-binding cassette (ABC) transporter, major facilitator superfamily (MFS), small multidrug resistance (SMR), resistance noduling division (RND), and (MATE) multidrug and toxic substance extrusion (Sun *et al*., 2014). Mostly RND efflux pump systems [47]. Multidrug resistance in *P. aeruginosa* belongs to three

protiens make up them: periplasmic linker-proteins [48], outer membrane protien (porine channales), and inner membrane transporters). Twelve RND-efflux systems produced by *P. aeruginosa* four of which resist antibiotics including MexAB, MexCD, MexEF, and MexXY [49]. From bacterial cells, MexAB is regulated to effluent the quinolone and lactam; MexCD, MexEF, and Mex XY have the potential to extrude B-lactam, quinolones, and aminoglycosides, respectively [50, 51, 52]. We found that the overexpression of various efflux pumps in specific clinical strains of *P. aeruginosa* increased the bacterial multidrug resistances [53].

1. **Antibiotic inactivating enzyme**

One important inherent resistance strategy in bacteria is the synthesis of enzymes that change or degrade drugs. Several antibiotics containing chemical linkages, like esters, and amides that are degraded by enzymes like β-lactamases and enzymes that modify aminoglycosides are usually generated by *P. aeruginosa* [54, 55]. The inducible beta-lactamase gene (AmpC), observed in *P. aeruginosa* and other GN bacteria, encodes the beta-lactamase enzyme. This enzyme disrupts the amide linkage of the beta-lactam structure, making beta-lactam drugs ineffective [56].

Additionally, relative to their peptide motifs, beta lactamases enzymes can be divided into four classes: A, B, C, and D. The active serine sites in classes A, C, and D enzymes permit them to hydrolyze ß-lactams. In contrast, class B Metallozymes need bivalent Zn cations for beta lactam hydrolysis [57]. *P. aeruginosa* produces class C ß-lactamase, which is designed to block antipseudomonal cephalosporin [58]. Extended-spectrum β-lactamase enzymes (ESBLs), which are created by specific clinical strains of *P. aeruginosa*, greatly boost resistance to primary beta lactam antibiotics like cephalosporins, penicillins, and aztreonams [59, 60]. Many variable factors including enzyme alteration, ribosomal modification cause by 16SRNA, efflux pump systems, and variation of cell membrane permeability play a role in aminoglycoside resistance in *P. aeruginosa* bacteria. According to Ramirez and Tolmasky [61], bacteria possess three unique types of aminoglycoside alteration enzymes: aminoglycoside nucleotidyltransferase (ANT), aminoglycoside phosphotransferase (APT), and aminoglycoside acetyltransferase (AAC). Gentamicin, kanamycin, tobramycin, netilmicin, and amikacin have all been demonstrated to be inhibited by the class one (ACC) of *P. aeruginosa* inactivation enzyme Poole *et al*., [54] mentions that at aminoglycoside sites 3′ and 6′, this enzyme acetylates the amino group to accomplish this. These aminoglycosides can resist tobacco smoke, gentamicin, and amikacin by adenylylation of amino or hydroxyl groups [62]. However, it also works against tobacco smoke. Research indicates that aminoglycosides like kanamycin, streptomycin, and neomycin are blocked from working by the APHs *P. aeruginosa* suppression enzyme, which binds a phosphate group to their 3′-hydroxyl [63, 64].

4.2 Acquired Resistance

Acquisition of foreign genes is a control to antibiotic resistance of *P. aeruginosa*, by lateral gene transfer and change in genetic locus, that leads to acquired resistance; this resistance also be acquired from mutations that inhibit multiple antibiotic classes, such as aminoglycosides, beta-lactams, and fluoroquinolones [65]. Chromosomal mutations in topoisomerase IV structural genes and DNA topoisomerase II that act as common pathways of acquired resistance to fluoroquinolones [66]. Another type of aquired resistance involved increased mutations of target sites, reduced antibiotic permeability, efflux pumps, and the generation of antibiotic-alteration enzymes. Such mechanisms induce chemical modification in the antibiotic or lead to its degradation [65]. Prevalent antibiotic-alteration enzymes encompass aminoglycoside-alteration enzymes (such as aminoglycoside acetyltransferases, aminoglycoside nucleotidyltransferases, and aminoglycoside phosphoryl transferases), acquired β-lactamases, carbapenemases, extended-spectrum β-lactamases (ESBLs), 16s rRNA methylases, and enzymes implicated in LPS modifications [67].

4.3 Adaptive resistance

Adaptive resistance strategy boosts the capability of a microbe to survive in the present antibiotics in the medium due to temporary alterations in gene and protein expression in response to an environmental stimulus, and it is reversible upon removal of the stimulus [68]. In *P. aeruginosa*, the first distinctive mechanisms of adaptive resistance are biofilm generation and the formation of persisted cells, which causes long-term infection and poor identification in CF individuals [69].

1. Resistance mediated by biofilm formation and quorum sense system

Thi *et al*., [10] emphasize that biofilms of *P. aeruginosa* are distinguished by a complex matrix formed from many polymeric substance network, involving extracellular-DNA (eDNA), lipids, polysaccharides, proteins. Moreover, known structural integrity to the biofilm, this matrix has many substantial profits for the bacteria inhabiting it, such as biofilms acting as a physical barrier versus the immune system of the host [70]. Bacteria that have biofilms exhibit notably greater resistance to antibiotics and antimicrobial agents through decreased antibiotic penetration and metabolic activity [71]. Water kept by biofilms can aid bacterial survival in stress or arid conditions. This feature supports biofilm communities [72]. Biofilms enable efficient retain and absorption of nutrients, so develop bacterial growth via raised enzyme activity that further aids in resource optimization. Biofilms also aid bacteria in adhering to infection sites and facilitate spread infection [73].

Quorum signaling is a communication action between microbial cells that coordinate their gene expression based on the density of populations [74]. *P. aeruginosa* have three key quorum signaling systems, including Rhl, PQS, and Las systems, all of which participate in biofilm production [75]. PA14 strain of P. *aeruginosa* has GacA-deficient displayed a tenfold decreasing in the ability of biofilm generation in contrast to the PA14 wild-type strain, indicating a critical role of the GacS/GacA regulatory system in the generation of biofilm [76]. On the other hand, the RetS kinase in the RetS/LadS regulatory system suppressed biofilm production in *P. aeruginosa* [77]. Moreover, eDNA released by damaged cells is other significant biofilm component part, which assists primary cell-cell interaction and accumulation on surfaces [78] *P. aeruginosa* undergoes a lot of physiological variation through biofilm generation. In constant CF infections, *P. aeruginosa* demonstrates a mucoid phenotype featured by promoted production of alginate. Furthermore, *P. aeruginosa* originally depend on flagellum-mediated for motility but reduce flagellar gene expression after adhering to surfaces to avoid immune system recognition. Modern research has exposed critical observations into the potent antibiotic resistance action operating within *P. aeruginosa* biofilm. Sadovskaya *et al*., [79] recognize a class of cyclic glycerophosphorylated β-1,3-glucans produced by *P. aeruginosa* in the extracellular part of biofilms. These β(1,3) glucans were established to to react with kanamycin antibiotics. Additionally, research has found novel genes identified in the clinical PA14 *P. aeruginosa*. However, these genes have a simple direct effect on biofilm development; they significantly boost biofilm drug resistance. The ndvB gene encodes a glucose transferase that synthesizes periplasmic (1,3)-linked glucans, which physically reserves tobramycin by preventing its binding to the target site. In the LasR/LasI, LasI synthesizes the substance 3-oxo-C12:2-HSL. The QS action is dependent upon this chemical [80]. It links to LasR, which expresses LasI and creates pathogenicity factors. It also contributes to the generation of biofilm. Whereas, the RhlR/RhlI signaling system consists of RhlI, which generates N-butyryl-L-homoserine lactone (C4-HSL) signaling substance. Its catalysis RhlI by binding to RhlR, that way initiating the expression of virulence genes and biofilm. The Las and Rhl systems work in conjunction, regulated by a protein indicated to as the *Pseudomonas*-Quinolone signaling system (PQS) [81]. Understanding the complex QS system in *P. aeruginosa* boosts the realization of the bacteria mechanisms for evolving antibiotic resistance and inducing disease.

Conclusion

Pseudomonas aeruginosa act as a critical threat in both healthcare and environmental habitat due

to its diverse virulence factors and notable capability to develop antibiotic resistance. Its varied range of pathogenic mechanisms, such as the biofilm formation, production of toxins, and the secretion of enzymes, enables it to develop persistent infections in immunodeficiency individuals. Furthermore, the bacterium's inherent, adaptive, and acquired resistance to several classes of antibiotics, facilitated by efflux pumps, modified drug targets, and enzymatic degradation, makes treatment strategies increasingly challenging. As antibiotic resistance continues to grow, new treatments and prevention strategies are urgently needed to manage infections caused by *P. aeruginosa*.

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