Updates on the pathogenicity status of *Pseudomonas aeruginosa*

Mohd W. Azam and Asad U. Khan

Medical Microbiology and Molecular Biology Laboratory, Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh 202002, India

Pseudomonas aeruginosa **is a pathogenic bacterial species that causes infections and diseases in both plants and animals, including several human diseases, especially in immune-compromised patients, and many hospital-acquired infections. Given that *P. aeruginosa* is an opportunistic pathogen, the occurrence of antimicrobial resistance makes it difficult to treat and eradicate. Antimicrobial resistance in *P. aeruginosa* is categorized as intrinsic, acquired, or adaptive. Here, we different aspects of resistance and pathogenicity in *P. aeruginosa*, such as the role of outer membrane proteins, transcriptional regulators, efflux pumps, enzymes, and biofilms in antimicrobial resistance. We also highlight quorum-sensing (QS) genes, their protein secretion, and role in pathogenicity; different QS inhibitors; and the influence of QS on the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas system and virulence factor production.**

Introduction

Pseudomonas aeruginosa is a motile (with a single polar flagellum), Gram-negative, bacillus (rod-shaped), aerobic, nonspore-forming opportunistic pathogenic bacteria that belongs to the *Pseudomonadaceae* [1]. *P. aeruginosa* occurs in both abiotic and biotic environments, from soil and aquatic environments to plant and animal tissues. It can be isolated from various sources, including: several nosocomial and life-threatening infections in patients with cystic fibrosis (CF), burn wounds, urinary tract infections (UTIs), and pulmonary infections; from the medical equipment, such as inhalers, dialysis equipment, respirators, anesthesiology equipment, and vaporizers; and from toilets and sinks [2]. *P. aeruginosa* is a pathogenic organism that causes disease both in plants and animals, including humans and is a major cause of hospital-acquired infections (HAIs) in patients. It causes hospital-acquired pneumonia (HAP) along with ventilator-associated pneumonia, gastrointestinal infections, dermatitis, UTIs, skin infections, such as folliculitis and external otitis, bacteremia, soft tissue infections, respiratory system infections in patients with CF, bone and joint infections, and several other infections especially in patients with severe burns, and immunocompromised patients, such as those with cancer or AIDS [3,4].

P. aeruginosa has evolved antimicrobial resistance, making it difficult to treat and limiting our therapeutic options. Some *P. aeruginosa* strains are resistant to most of the available antimicrobial agents, from carbapenem to the third-generation cephalosporins, which are the preferred options for treating multidrug-resistant (MDR) bacteria [5].

MDR *P. aeruginosa* is frequently isolated from patients with CF, patients with neoplasia, and also in isolated outbreaks in intensive care units (ICU) [4]. More than 51 000 healthcare-associated infections and around 440 deaths caused by *P. aeruginosa* are reported annually in the USA. Of these infections, >13% (~6700) are MDR *Pseudomonas*. The US Center for Disease Control Antibiotic Resistance (CDC AR) threat report classifies MDR *P. aeruginosa* as a serious threat [6]. The 2017 WHO list of antibiotic-resistant priority pathogens, a catalog of 12 bacterial families that are classified as the greatest threat to the human health, lists carbapenem-resistant *P. aeruginosa* as one of the most critical threats (Fig. 1) [7]. The *Antimicrobial Resistance Surveillance in Europe 2015 Report* indicated that, in most European countries, *P. aeruginosa* resistance is common (>10% of outbreaks), that 13.7% of *P. aeruginosa* isolates were

Corresponding author: Khan, A.U. (asad.k@rediffmail.com)

https://doi.org/10.1016/j.drudis.2018.07.003

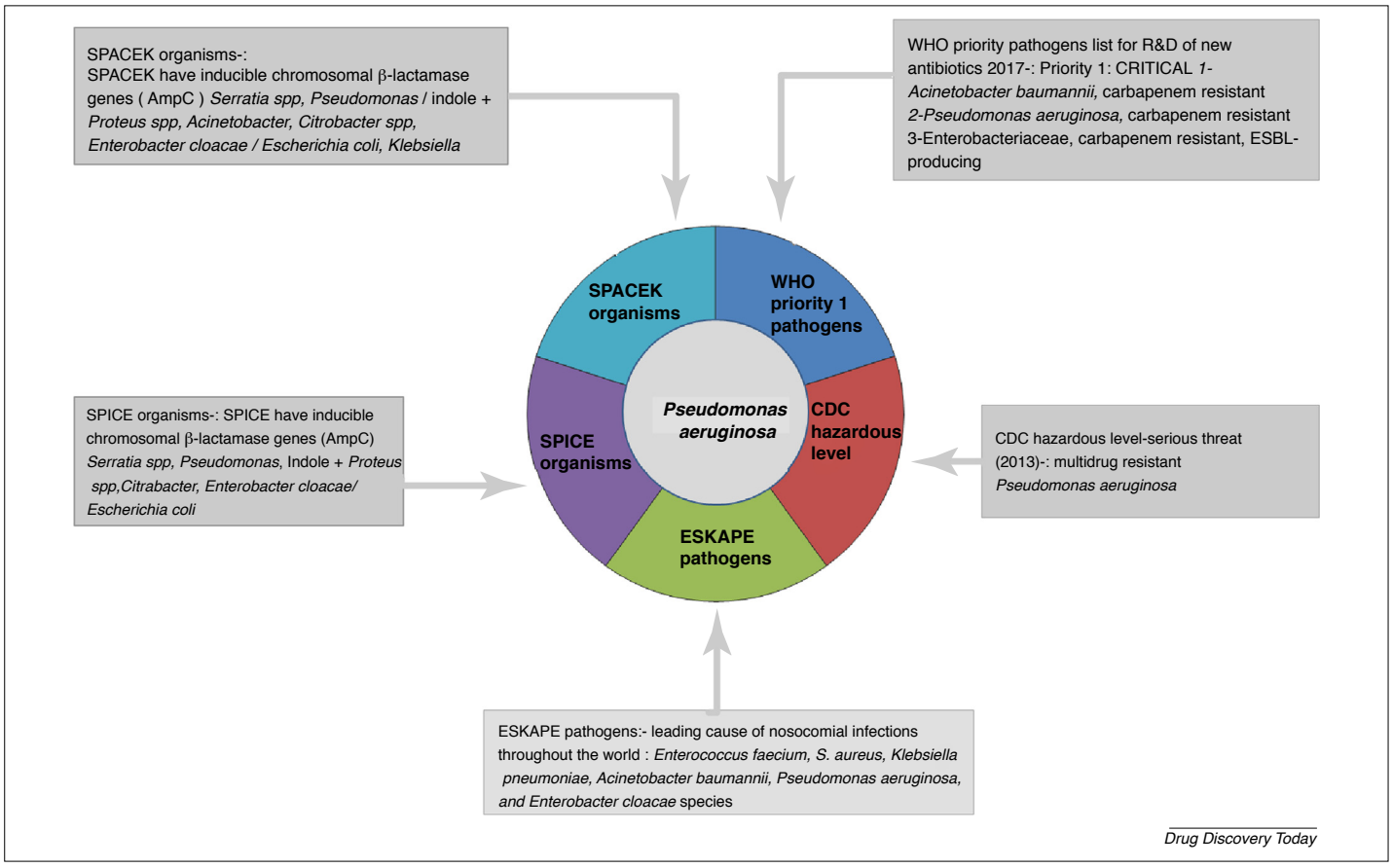

FIGURE 1

Mnemonics assigned to *Pseudomonas aeruginosa*, based on pathogenicity. SPICE and SPACE organisms, which include Gram-negative bacteria, have inducible, chromosomal β-lactamase genes (AmpC). ESKAPE pathogens are the leading cause of life-threating nosocomial infections and have antimicrobial resistance genes on chromosomes, plasmids, or transposons. The US Center for Disease Control (CDC) classifies *P. aeruginosa* as a serious threat, whereas WHO flagged it as a priority one pathogen.

resistant to at least three antimicrobial groups, and that 5.5% of *P. aeruginosa* isolates were resistant to all five antimicrobial groups, as reported following regular European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance [8].

P. aeruginosa has evolved multiple mechanisms to resist antimicrobial agents (Fig. 2). Antimicrobial resistance in *P. aeruginosa* can be divided into intrinsic resistance, acquired resistance. and adaptive resistance [9]. Given the presence of both intrinsic and acquired modes of resistance in *P. aeruginosa*, it is difficult to cure any resulting infections.

Intrinsic resistance

Intrinsic resistance is the ability of a microorganism to resist antimicrobial agents. Opportunistic pathogens (e.g., *P. aeruginosa*) have high intrinsic resistance to various antimicrobial agents [10]. The intrinsic antimicrobial resistance in *P. aeruginosa* involves several mechanisms, including a decrease in outer membrane permeability, expression of MDR efflux pumps, and production of antibiotic-inactivating enzymes [11]. The permeability barrier of the outer membrane prevents antimicrobials penetrating the bacterial cells. This decreased membrane permeability occurs mainly because of the alteration of outer membrane porin proteins, such as OprD, major proteins targeted by carbapenems [12].

Bacterial cells synthesize enzymes that selectively target antibiotics and inactivate them via chemical alterations, such as the addition of specific chemical moieties or the complete destruction of the antibiotic molecule [13,14]. Antibiotic-inactivating enzymes confer resistance against antimicrobials either by hydrolyzing the antibiotics, such as the expression of chromosomally encoded AmpC β-lactamases, which hydrolyze most β-lactams, and carbapenem-hydrolyzing enzyme PoxB [15]. Enzyme-dependent resistance involves both plasmid- and chromosomal-encoded enzymes and the production of these enzymes might result from the development of acquired or intrinsic resistance.

Bacterial efflux pumps allow microorganisms to resist antimicrobial agents and regulate the internal cell environment by pumping out harmful agents (e.g., antibiotics, metabolite etc.) from the cell interior. Efflux pumps in *P. aeruginosa* belong mainly to the resistance nodulation division (RND) family, which are tripartite multidrug efflux systems. Tripartite efflux systems are divided into the outer membrane channel, periplasmic adapter protein, and inner membrane transporter [16]. Such pumps contribute to both adaptive and acquired resistance in *P. aeruginosa* through proton-dependent expulsion (mainly) of antimicrobial agents and several other harmful agents from the cell [17]. Other reported pump families include multidrug and toxic compound extrusion (MATE), major facilitator superfamily (MFS), and small

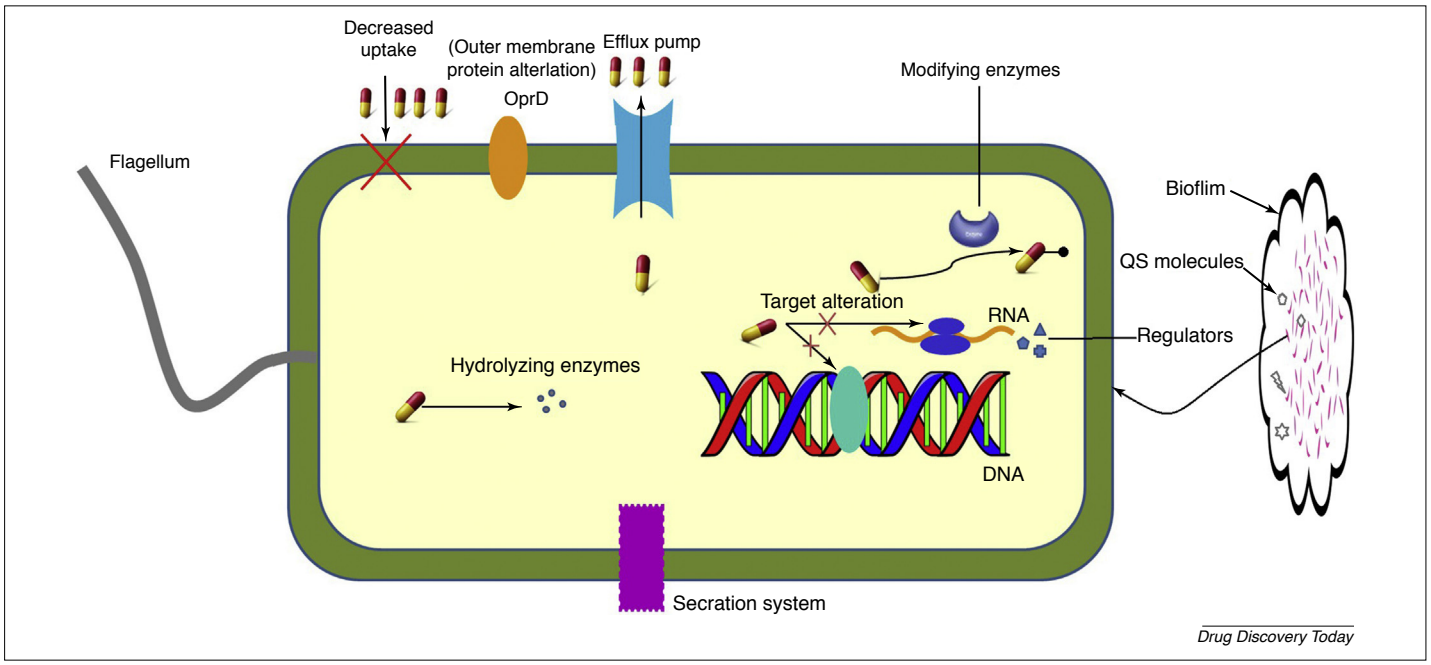

FIGURE 2

Mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*. Outer membrane protein (OprD) alteration causes a change in membrane permeability. Drug-hydrolyzing enzymes hydrolyze drugs, whereas drug-modifying enzymes modify drugs, rendering them inactive. Some enzymes cause target alterations such that the drug will unable to bind its target or the expression of genes involved in efflux pumps increased, resulting in the drugs being pumped from the cells. Biofilms act as physical barriers and prevent antibiotics penetrating the cell. Secretion systems secrete several proteins that help cell survival and pathogenicity. Abbreviation: QS: quorum sensing.

multidrug resistance (SMR) families [18]. In wild-type *P. aeruginosa* strains, a constitutively expressed efflux pump, MexAB-OprM, has a role in intrinsic resistance to most β-lactams and many other antimicrobials [19]. The MexXY-OprM pump is also involved in the intrinsic resistance of *P. aeruginosa* to tetracycline, erythromycin, and gentamicin. Efflux pumps have conferred resistance against several antimicrobials, including chloramphenicol, macrolides, erythromycin, roxythromycin, lincosamides, ketolides, glycylcyclines, and aminoglycosides [20].

Acquired resistance

The development of acquired resistance in *P. aeruginosa* can result from the acquisition of external genes responsible for resistance through horizontal gene transfer and chromosomal gene mutations. Acquired resistance resulting from mutations imparts resistance against several classes of antibiotic, including β-lactams, fluoroquinolones (FQs), aminoglycosides, and polycationic antimicrobials [13]. One the most frequent mechanisms of acquired resistance to FQs is chromosomal mutations in the genes encoding subunits of the enzymes DNA gyrase (gyrA-gyrB) and topoisomerase IV (parC-parE) [21]. Additional mechanisms of resistance include mutational upregulation of efflux pumps; decreased permeability to antibiotics; target site alterations through mutations; and antibiotic-modifying enzyme production. Such mechanisms result in the chemical alteration of the antibiotic or its destruction. The production of enzymes causing chemical alteration of antibiotics is an important mechanism of acquired resistance. The most common types of such alterations are phosphorylation (chloramphenicol and aminoglycosides), acetylation (chloramphenicol, streptogramins, and aminoglycosides), and adenylation (aminoglycosides and lincosamides) [13]. Common antibiotic-modifying enzymes include aminoglycoside-modifying enzymes (e.g., aminoglycoside acetyltransferases, aminoglycoside nucleotidyltransferases, aminoglycoside phosphoryl transferases), acquired β-lactamases, carbapenemases, extended-spectrum β-lactamases (ESBLs), 16s rRNA methylases, and those involved in lipopolysaccharide (LPS) modifications [8,12,22].

Adaptive resistance

Adaptive resistance is an inducible resistance that occurs in response to the presence of antimicrobial agents (e.g., antibiotics) or other environmental stresses (chemical or physical), such as a change in media, pH, temp, oxygen, and other growth conditions [23]. In contrast to adaptive resistance, intrinsic and acquired resistance are characterized by an irreversible phenotype and are independent of the presence of antibiotics and environmental stimulus [24]. In addition to antibiotics, several environmental factors, such as heat shock, DNA stress (SOS response), polyamines, nutrient deficiencies, biocides, anaerobiosis, cation levels, and changes in carbon sources, and social behaviours, such as biofilm formation and swarming motility, can trigger adaptive resistance [23–25]. *P. aeruginosa* is a highly adaptable bacterium that can change its response according to its environment [9]. It has one of the largest bacterial genomes (6.3 Mbp, encoding 5567 genes) compared with that of *Escherichia coli* (4.6 Mbp, 4279 genes), *Bacillus subtilis* (4.2 Mbp), *Mycobacterium tuberculosis* (4.4 Mbp), *Staphylococcus aureus* N315 (2.81 Mbp, 2594 genes), *Haemophilus influenzae* Rd (1.83 Mbp, 1714 genes), and *Synechocystis* (3.6 Mbp) [9,26]. Manual annotation of the *P. aeruginosa* genome calculated 521 regulatory genes (9.4% of the total genes), which encode

either transcriptional regulators or two-component regulatory system proteins. Pfam 5.2 family models and HMMER 2.1.1 (http://hmmer.wustl.edu/) analysis reported 468 regulatory genes (8.4%) in *P. aeruginosa*. The regulatory gene potential of *P. aeruginosa* is high compared with other bacterial species (*E. coli* 5.8%; *B. subtilis* 5.3%; *M. tuberculosis* 3%; and *Helicobacter pylori* 1.1%) [26]. It has been estimated that ~1500 genes are required for cell division and growth in a minimal salt medium that also includes all structural proteins and metabolic enzymes. These data suggest that *P. aeruginosa* has a considerable reserve (additional) genetic capacity compared with other bacterial species, and also explain the highly adaptable nature of *P. aeruginosa*, including resistance against antimicrobials and other environmental stresses [9].

Many regulators in *P. aeruginosa* are involved in both antibiotic resistance and virulence (Table 1). Depending on the type of regulator, regulators might have a role in intrinsic, acquired, or adaptive resistance [23].

Pseudomonas aeruginosa biofilms and resistance

P. aeruginosa is a model organism for the study of biofilms. Bacterial cells growing as biofilms show many properties that differ from planktonic cells, such as enhanced resistance to antimicrobials, and can also act against the host immune system [27]. *P. aeruginosa* can exist in both biofilm and planktonic forms. Biofilms of *P. aeruginosa* comprise extracellular polymeric (EPS) matrix-enclosed cells. This matrix contains polysaccharides, lipids, proteins, and nucleic acids (extracellular DNA or eDNA and RNA) and biosurfactants, which aid the initial adherence of cells, and provide structure, to the biofilm [28]. *P. aeruginosa* cells produce matrix when the cells show a high degree of autoaggregation during the later stages of biofilm development [28]. The exopolysaccharides Pel, Psl, and alginate are produced by *P. aeruginosa* and are involved in biofilm formation [29]. Cyclic di-GMP, a nucleotide signaling molecule (bacterial second messenger), is a key regulator of EPS production, switching of bacterial cells from motile to non-motile stages (sessile), and of adherent cells in biofilms [25,30]. *P. aeruginosa* biofilm formation involves four main steps: attachment to a surface (biotic or abiotic), multiplication, microcolony formation, and maturation into a structured and resistant microbial community [31]. Biofilms provide another way of escaping antimicrobial agents, and makes it difficult to treat any resulting infections [32].

Pseudomonas aeruginosa *biofilms confer resistance to antimicrobials*

The biofilm exopolysaccharide matrix acts as a diffusive barrier, preventing the diffusion of antimicrobials as well as immobilizing antibiotics [23,33]. In addition, diffusive barriers are also responsible for the generation of nutrient gradients, which can result in a decline in growth rate and a reduction in metabolic activity, leading to an increase in persister cells [34]. Given differences in the availability of nutrients and oxygen, the growth of bacterial cells varies in different regions of the biofilm. Bacterial cells growing on the biofilm surface are more metabolically active, whereas inner cells grow more slowly. Most β-lactams and aminoglycosides are effective only against growing cells, whereas other antibiotics, such as polymyxins, kill cells with poor growth condition. This is why different antibiotics act on different regions of biofilms [23,33]. Low concentrations of antibiotics kill most *P. aeruginosa* biofilm cells but increasing their concentration does not kill persister cells. Thus, researchers have concluded that only a small fraction of the biofilm cell is responsible for its enhanced antibiotic resistance and that most biofilm cells are susceptible to antibiotic treatment, similar to planktonic cells [33,35]. Gram-negative bacterial biofilms show more persister cells compared with other biofilms because their cell walls comprise LPS, which further enhances the nonpenetration of antibiotics into the cells [34].

Bacterial cells in the biofilm undergo several genetic, metabolic, physiological and phenotypic changes [33,36]. In *P. aeruginosa*, colonies obtained from biofilms can appear as dwarf, mucoid, hyperpiliated, LPS-deficient, rough, wrinkled, or antibiotic-resistant colonies [36]. Exopolysaccharide Pel overproduction is associated with the wrinkled colony formation, whereas overproduction of alginate is associated with finger-like microcolony formation. Alginate production reduces biofilm susceptibility to antibiotics as well as to human immune defence mechanisms [37].

P. aeruginosa biofilms are common in patients with CF pneumonia, UTIs, and in medical devices contact lenses, and catheters

TABLE 1

Regulatory elements of *P. aeruginosa* involved in virulence and antibiotic resistance.

Regulator (gene)	Description	Function	Refs
BrlR (*brlR*)	Biofilm resistance locus regulator	Hydrogen peroxide tolerance; has role in antimicrobial resistance gene activation	[54]
Crc (*crc*)	Catabolite repression control, global regulator	Controls carbon source metabolism and catabolite repression, secretion system, motility, antibiotic resistance	[55]
PsrA	Type III transcriptional regulator	Biofilm formation, swarming, intrinsic and adaptive resistance	[23]
CbrAB	Two-component regulatory system	Acquired resistance, swarming, and biofilm development	[23]
PA0756-PA0757	Encodes two-component response regulator	Resistance against tobramycin and gentamicin	[56]
PhoPQ	Two-component regulatory system	Swarming, biofilm formation, twitching, and antimicrobial peptide resistance under low Mg^{2+} concentrations	[23]
Lon	ATP dependent protease	Biofilm formation, swarming, intrinsic and adaptive resistance	[23]
phoB	Part of PhoB-PhoR two-component signaling system	Sense phosphate scarcity and consequently turns on *vreA*, *vreR*, and *vreI* expression	[57]
PmrAB	Two-component regulatory system	Involved in resistance to cationic antimicrobial peptides, including polymyxins	[58]
AmpR	Global regulator	Affects expression of multiple genes (e.g., *ampC*, *lasA*, *lasB*, *lasI*, *rhlR*, and *poxB*)	[59]

[3,37]. *CFTR* gene mutations cause defects in chloride ion channels, which leads to CF, an autosomal recessive genetic disorder [38]. *P. aeruginosa* forms dense and robust biofilms in the lungs of patients with CF and adapts well to the lung environment, which is unaffected by intensive antibiotic treatment and the inflammatory responses of the host in the form of polymorphonuclear leucocytes (PMNLs). CF can lead to respiratory failure, lung transplantation, or death [37,38]. The lungs of patients with CF are invaded initially by nonmotile and mucoid phenotype *P. aeruginosa* strains, followed by selection of few phenotypes during the course of infection. The selected phenotypic variants are nonmotile, rough LPS in texture, and mucoid because of alginate overproduction [39].

Quorum sensing and virulence factors

QS is a cell density-dependent intercellular bacterial communication system in bacteria that enables the individual cells to act as a community. QS performs an important role in bacterial virulence, resistance, and biofilm formation. The expression of more than 300 genes in *P. aeruginosa* is controlled by QS [40]. In *P. aeruginosa*, there are currently four known interconnected QS communication systems: las, rhl, *Pseudomonas* quinolone signal (pqs), and iqs [41]. Las and rhl are *N*-acylated homoserine lactone (acyl-HSL) signaling systems. The las system comprises an autoinducer synthase, lasI, which produces the autoinducer *N*-3-oxo-dodecanoyl-homoserine lactone (3OC12-HSL). By contrast, the rhl system has an autoinducer synthase, rhlI, which produces *N*-butanoyl-homoserine lactone (C4-HSL). The third system, pqs, involves an autoinducer synthase, PqsABCDH, which produces 2-heptyl-3-hydroxy-4-quinolone signals or simply quinolone. The final system is iqs, which has an autoinducer synthase, AmbBCDE, which produces 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS). The receptors for las, rhl, and pqs are LasR, RhlR, and PqsR, respectively [42–44]. The iqs receptor has yet to be fully determined. Inducer 3-oxo-C12-HSL of the rhl system binds to LasR and activates lasI and several downstream genes. This complex (LasR- autoinducer) also activates expression of rhlR and rhlI genes of the rhl system as well as pqsR and pqsABCDH genes of the pqs system. The PQS system regulates las and rhl QS through 2-heptyl-3-hydroxy-4-quinolone production [44]. Under normal bacterial growth, IQS production is firmly controlled by the las system. IQS production can also be activated by phosphate starvation, generally during bacterial infection. IQS is an essential part of the *P. aeruginosa* QS mechanisms that control its virulence and physiology. IQS regulates the expression of a large number of genes, many of which are QS-associated genes as well as virulence factor-related genes [45].

Several virulence factors are produced by *P. aeruginosa* during various growth stages that might or might not be regulated by QS genes (Fig. 3 and Table 2).

Secretion system in *Pseudomonas aeruginosa*

Gram-negative bacteria have a double cell membrane with a complex system of protein secretion. These secreted products help bacteria to survive in eukaryotic hosts by avoiding the host immune system, allowing them to cause infections. The secreted products can be an enzyme that causes enzymatic hydrolysis of complex compounds (e.g., carbon sources) into simpler compounds that the bacterial cell can utilize, or a protein assisting in the uptake of essential ions [46]. These secreted proteins have several important roles in antimicrobial resistance, disintoxication, and scavenging [47]. Many of these secreted proteins act as virulence factors. In *P. aeruginosa*, there are multiple secretory pathways that are responsible for the release of several proteins, toxins, and enzymes to the cell exterior [48]. Currently, there are nine types (T1SS to T9SS) of secretion systems reported in prokaryotes [46,49]. Of these systems, only five have been reported in *P. aeruginosa* (T1SS, T2SS, T3SS, T5SS, and T6SS).

Inhibitors of QS and virulence factors

P. aeruginosa has nearly all the known mechanisms of antimicrobial resistance. The occurrence of such resistance has triggered the search for novel antimicrobial drugs against alternative drug targets. Several strategies and targets have been adopted by researchers to counter *P. aeruginosa* infections. For example, inhibitors against efflux pumps and QS were found to be effective against P. *aeruginosa* infection (Table 3). These inhibitors can be either isolated from a natural source or synthesized.

Antiactivator proteins are known to suppress QS activation through R-proteins. There are three antiactivator proteins (QteE, QscR, and QslA) reported in *P. aeruginosa*. QteE forms a heterodimer with LasR, preventing signal binding, destabilizing LasR, and reducing RhlR QS-transcriptional activity, as well as destabilizing the RhlR protein. QscR suppresses QS-regulated operons involved in hydrogen cyanide and phenazine biosynthesis, whereas QslA forms a heterotrimeric complex and works as a strong antiactivator of LasR [50].

Pseudomonas and the CRISPR-Cas system

CRISPR along with CRISPR-associated (Cas) proteins confers an adaptive immune system that occurs in both Archaebacteria and Eubacteria. CRISPR is a sequence-specific adaptive immune defence mechanism against foreign DNA, which can be in the form of plasmid DNA, bacteriophage DNA, or transposons [51,52]. The CRISPR-Cas system (type 1-F) of *P. aeruginosa* has an important role in various alternative functions, such as biofilm inhibition, loss of swarming motility, and phage resistance [51,53]. In *P. aeruginosa*, QS activates CRISPR-Cas dependence mechanism and, therefore, QS inhibitors could be used as anti-CRISPR-Cas adaptations (i.e., acquisition of resistance against the administered phage) [52]. This inhibition of CRISPR-Cas defence mechanisms with the help of QS inhibitors would lead to *P. aeruginosa* being more susceptible to killing via the use of phage therapy.

Concluding remarks

P. aeruginosa is an opportunistic pathogen involved in many life-threating infections, including those associated with CF and hospital-acquired infections. The extensive and indiscriminate use of antibiotics has led to the evolution of antibiotic resistance. Antimicrobial resistance in *P. aeruginosa* can be adaptive, intrinsic, or acquired. Efflux pumps, transcription regulators, and antibiotic-inactivating enzymes have important roles in antibiotic resistance. Biofilm production gives an additional advantage to antimicrobial resistance by lowering the accessibility of antimicrobial agents to the cells. QS also has a crucial role in bacterial virulence, biofilm formation, and antibiotic resistance. Several genes associated with

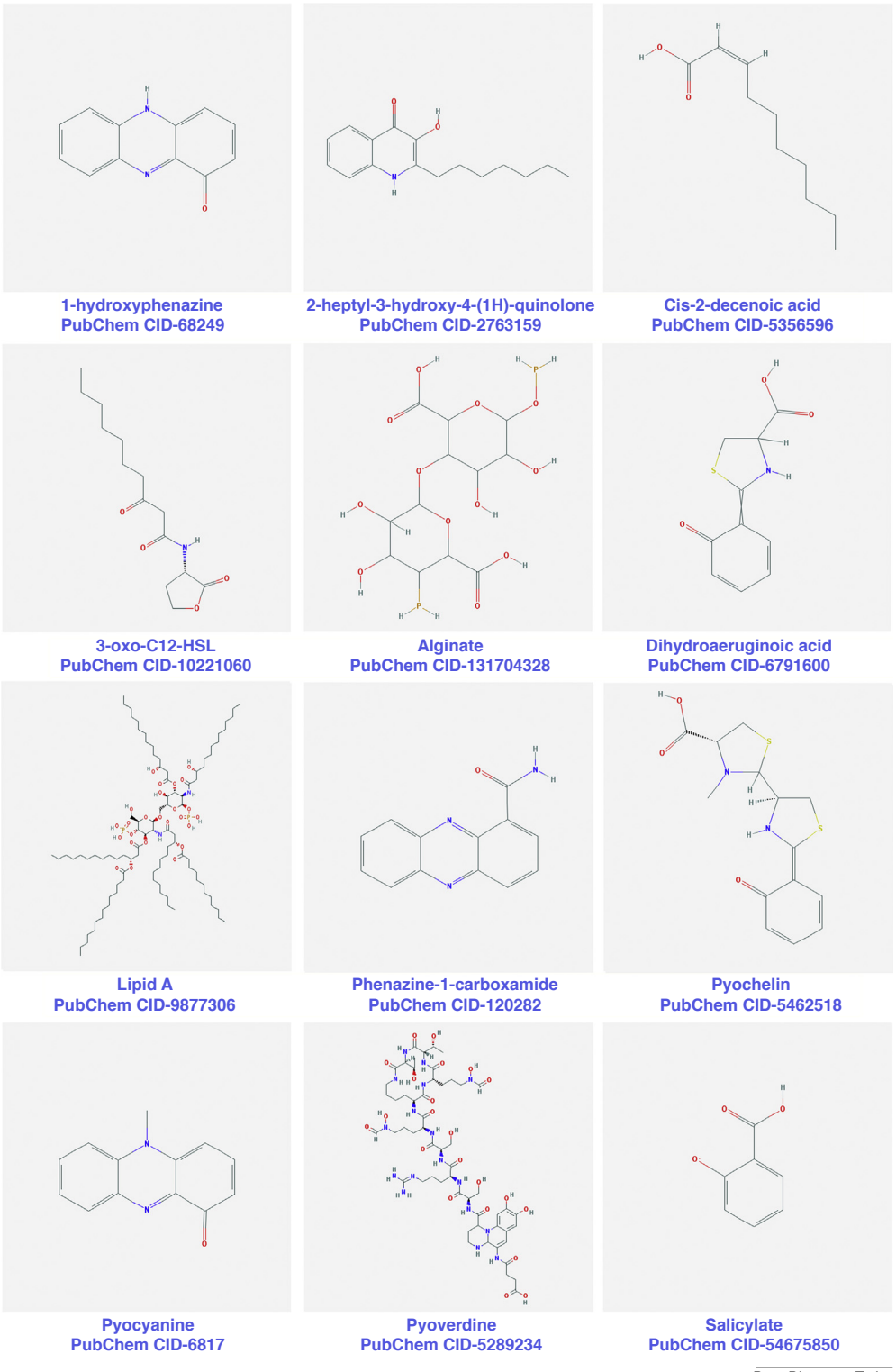

FIGURE 3

Structure of virulence factors produced by *Pseudomonas aeruginosa* [104]. Some of these virulence factors might act as quorum sensing signal molecules.

TABLE 2

***Pseudomonas aeruginosa* virulence factors and associated genes**

Category	Virulence factor	Gene (s) involved	Description	Refs
Exopolysaccharides	Alginate	Three chromosomal regions: (i) algR (algR1), algQ (algR2), and algP (algR3) at 9 min; (ii) algB at 13 min; (iii) algU (algT), mucA (algS), and mucB (algN) at 68 min	Finger-like microcolony formation, biofilm antibiotic susceptibility reduction	[20,60]
	Psl	PSL gene cluster contains 15 co-transcribed genes (pslA to pslO)	Initial attachment and biofilm maturation	[20,61]
	Pel	PEL gene cluster (pelA to pelG)	Wrinkled colony phenotype, cell–cell interaction, role in biofilm cell density and/or biofilm compactness	[20,62]
LPS	A-band-O-antigen	A-band gene cluster	Common antigen, expressed by most serotypes; contains homopolymer of a-D-rhamnose	[63,64]
	B-band-O-antigen	WBP cluster	Serotype specific; contains heteropolymeric O antigen	[63,65]
	Lipid A	Lipid A modifications in *P. aeruginosa* regulated by two-component regulatory systems PhoP/PhoQ and PmrA/PmrB	Bioactive component of LPS, pathogenic factor	[66]
Phenazines	Phenazine-1-carboxylic acid	PhzA1-G1 and phzA2-G2 operons	Toxic to *Caenorhabditis elegans*	[67]
	Pyocyanin	PhzM, phzS, phzABCDEFG	Act as toxins against bacteria, fungi, and mammals	[67]
	1-hydroxyphenazine	PhzM	Toxic to *C. elegans* over a wide pH	[67]
	phenazine-1-carboxamide	PhzS	Toxic to *C. elegans*	[67]
	CFTR inhibitory factor (Cif)	PA14_26090 (cif locus)	Shows epoxide hydrolase (EH) activity, but sequence violates two conserved EH motifs	[68,69]
QS signal molecules	3-oxo-C12-HSL	Autoinducer synthase, lasI	Downregulates TNF-α production; contributes to establishment of chronic infection	[70,71]
	2- heptyl-3-hydroxy-4-(1H)-quinolone (PQS)	PqsABCDE operon	Bacterial cell autolysis at high population densities in nutrient-deficient conditions, iron-chelating properties, production of several virulence determinants	[70]
	2-heptyl-4(1H)-quinolone (HHQ)	PqsABCD and PqsE	Controls expression of many virulence genes as a function of cell population density	[70,72]
	2-(2-hydroxyphenyl)-thiazole-4- carbaldehyde (IQS)	AmbBCDE operon	IQS expression under unfavorable environment overcomes las-led QS circuit and promotes expression of virulence factors	[70]
	N-butanoyl-homoserine lactone (C4-HSL)	Autoinducer synthase rhlI	C4-HSL binds to RhlR and activates expression of genes required for production of a several secondary metabolites, such as HCN and pyocyanin	[70,73]
	cis-2-Decenoic acid (DSF)	DspI (PA14_54640, PA0745)	Biofilm dispersion autoinducer	[74]
Siderophores	Pyoverdine	Pvd genes	Iron-gathering, virulence	[75]
	Pyochelin	PchDCBA Operon, pchEFG	Chelator of transition metal ions [Fe(III), Zn(II), Cu (II), Co(II), Mo(VI), and Ni(II)]	[76,77]
	Salicylate	PchBA	Pyochelin biosynthetic pathway intermediate	[76]
	Dihydroaeruginoic acid	PchDCBA Operon	Antifungal antibiotic	[76]
Surfactant	Rhamnolipid	RhlAB	Maintains noncolonized channels surrounding macrocolonies	[78]
Exoenzymes	Elastase B	LasB	Elastolytic zinc metalloproteinase, necessary virulence factor for initial pathogenesis process, elastin degradation	[79,80]
	Elastase A	LasA	Secreted virulence factor responsible for shedding of host cell surface proteoglycan syndecan-1	[81]
	Protease IV	PrpL	Serine endoprotease, degrades plasminogen immunoglobulins, fibrinogen, and elements of mammalian complement immune system	[77,81]
	Exotoxin A	ToxA	Toxic virulence factor	[41,79]
	Exoenzyme S (ExoS)	ExoS gene	Antiphagocytic factor, ADP-ribosylating enzyme	[79,82]
	Alkaline protease	AprA	Zinc metalloprotease, colonization	[69,79]
	Protease	LasA	Elastin degradation shows high staphylolytic activity	[80]
	Hemolysin (phospholipases C)	PlcHR operon	Causes vascular permeability, end-organ damage	[83]

TABLE 2 (*Continued*)

Category	Virulence factor	Gene (s) involved	Description	Refs
Other	Hydrogen cyanide	HcnABC	Virulence factor, present in burn wounds infected with *P. aeruginosa* and *P. aeruginosa* CF isolates	[84]
	LecA Lectin (PA-IL)	LecA	Galactose binding, cytotoxic effects on respiratory epithelial cells, increases exotoxin A absorption by inducing permeability defect in intestinal epithelium	[85]
	LecB Lectin (PAIIL)	LecB	Fucose binding, involved in pilus biogenesis and protease IV activity	[86]
	Superoxide dismutase (Fe-SOD)	SodB	Iron-co-factored SOD protects cells from toxic effects of superoxide	[86]
	Superoxide dismutase (Mn-SOD)	SodA (sodM)	Manganese-co-factored SOD protects cells from toxic effects of superoxide	[86]
	Catalase	KatA, katB	Protects planktonic and biofilm cells against hydrogen peroxide	[87]

TABLE 3

***Pseudomonas aeruginosa* targets and their inhibitors**

Target	Inhibitor	Source	Description	Refs
Bacteriocin	Plantaricin DL3	Suan-Tsai (Chinese fermented cabbage)	Cell wall disruption and proteins leakage	[88]
Antibiofilm	Rhein	*Rheum palmatum* L.	Antibiofilm	[89]
	Chrysophanol	*Rheum officinale* Baill.	Antibiofilm	[89]
	Nodakenetin	*Peucedanum decursivum* (Miq). Maxim	Antibiofilm	[89]
	Shikonin	*Lithospermum erythrorhizon* Sieb.	Antibiofilm	[89]
	Emodin	Rheum palmatum L.	Antibiofilm	[89]
	Fraxin	*Fraxinus chinensis* Roxb.	Antibiofilm	[89]
	ε-Viniferin (Resveratrol dimer)	*Carex pumila*	Antibiofilm	[90]
	(z)-5-octylidenethiazolidine-2, 4-dione (TZD-C8)	Synthetic	Strong inhibitor of biofilm formation	[91]
QS inhibitors	*N*-(2-30 pyrimidyl) butanamide (C11)	Synthetic	Inhibits biofilm formation, downregulates las and rhl QS regulatory genes, and rhlA and lasB genes	[92]
	N-decanoyl cyclopentylamide (C10-CPA)	Synthetic	Inhibits lasB-lacZ and rhlA-lacZ expression, pyocyanin, elastase, and rhamnolipid production, and biofilm formation	[93]
	Azithromycin	Antibiotic	Reduces QS gene expression	[94]
	3-NH2-7Cl-C9-QZN	Synthetic	Inhibits alkyl-quinolone (AQ) signaling by antagonizing AQ biosynthesis, virulence gene expression, pyocyanin production, and biofilm development	[95]
	Meta-bromo-thiolactone (mBTL)	Synthetic	LasR and RhlR partially inhibited by mBTL, inhibits pyocyanin production and biofilm formation	[96]
	6-Gingerol	Ginger oil	Reduces biofilm formation and virulence factor	[97]
	M64	Synthetic	Competitive inhibitor of MvfR	[98]
Virulence factors inhibition	Compound 4	Synthetic	Inhibits pyocyanin production	[99]
	Flavonoids	Plant secondary metabolites	Suppresses virulence factor production and biofilm formation	[100]
Efflux pump	Phe-Arg-naphthylamide (PAβN or MC-207)	Synthetic	MDR efflux pump (RND efflux pump) inhibitor	[101]
LasB	*N*-mercaptoacetyl-Phe-Tyr-amide	Synthetic	Blocks LasB action, reduces biofilm growth	[102]
Other	Guanidinium-functionalized polycarbonates	Synthetic	Antibacterial activity against several pathogens, including *P. aeruginosa*	[103]

Pseudomonas QS are involved in virulence factor production as well as biofilm development. Many inhibitors have been designed against QS genes, but are losing their efficacy against MDR *Pseudomonas*. Therefore, there is an urgent need to develop alternative antimicrobial resistance therapies against such bacteria. Given that QS activates the CRISPR-Cas system in *Pseudomonas*, inhibition or interruption of this system could be an alternative approach to overcome MDR bacteria.

Acknowledgments

The authors are thankful to S E Hasnain for his critical review of this article. Amit Kumar is acknowledged for designing images.

References

1 Anon (2001) *Pathogen Safety Data Sheet Pseudomonas aeruginosa*. Montana State University
2 Gales, A.C. *et al.* (2001) Characterization of *Pseudomonas aeruginosa* isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular typing in the global SENTRY Antimicrobial Surveillance Program, 1997–1999. *Clin. Infect. Dis.* 15, 32
3 Todar, K. (2017) *Todar's Online Textbook of Bacteriology*. Publisher http://textbookofbacteriology.net/pseudomonas.html
4 Tacconelli, E. *et al.* (2002) Multidrug-resistant *Pseudomonas aeruginosa* bloodstream infections: analysis of trends in prevalence and epidemiology. *Emerg. Infect. Dis.* 8, 220–221
5 CDC (2018) *Pseudomonas aeruginosa in Healthcare Settings*. CDC
6 CDC (2018) *Antibiotic Resistance Threats in the United States, 2013*. CDC
7 WHO (2017) *WHO Publishes List of Bacteria for which New Antibiotics Are Urgently Needed*. WHO
8 ECDC (2015) *Antimicrobial Resistance Surveillance in Europe 2015*. ECDC
9 Lambert, P.A. *et al.* (2002) Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *J. R. Soc. Med.* 95, 22–26
10 Hall, C.W. *et al.* (2018) *Pseudomonas aeruginosa* biofilm antibiotic resistance gene ndvB expression requires the RpoS stationary-phase sigma factor. *Appl. Environ. Microbiol.* 84 e02762–17
11 Cox, G. *et al.* (2013) Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. *Int. J. Med. Microbiol.* 303, 287–292
12 Poole, K. *et al.* (2011) *Pseudomonas aeruginosa*: resistance to the max. *Front. Microbiol.* 2, 65
13 Munita, J.M. *et al.* (2016) Mechanisms of antibiotic resistance. *Microbiol. Spectr.* 4 http://dx.doi.org/10.1128/microbiolspec VMBF-0016-2015
14 Arzanlou, M. *et al.* (2017) Intrinsic, adaptive and acquired antimicrobial resistance in Gram-negative bacteria. *Essays Biochem.* 61, 49–59
15 Zincke, D. *et al.* (2016) Characterization of a carbapenem-hydrolysing enzyme, PoxB, in *Pseudomonas aeruginosa* PAO1. *Antimicrob. Agents Chemother.* 30, 936–945
16 Daury, L. *et al.* (2016) Tripartite assembly of RND multidrug efflux pumps. *Nat. Commun.* 7, 10731
17 Hassan, M.T. *et al.* (1999) Identification of a gene cluster, czr, involved in cadmium and zinc resistance in *Pseudomonas aeruginosa*. *Gene* 238, 417–425
18 Alav, I. *et al.* (2018) Role of bacterial efflux pumps in biofilm formation. *J. Antimicrob. Chemother.* Published online February 28, 2018. http://dx.doi.org./10.1093/jac/dky042
19 Okamoto, K. *et al.* (2001) *Pseudomonas aeruginosa* reveals high intrinsic resistance to penem antibiotics: penem resistance mechanisms and their interplay. *Antimicrob. Agents Chemother.* 45, 1964–1971
20 Poole, K. *et al.* (2005) Efflux-mediated antimicrobial resistance. *J. Antimicrob. Chemother.* 56, 20–51
21 Nouri, R. *et al.* (2016) The role of gyrA and parC mutations in fluoroquinolones-resistant *Pseudomonas aeruginosa* isolates from Iran. *Braz. J. Microbiol.* 47, 925–930
22 Henrichfreise, B. *et al.* (2007) Resistance mechanisms of multiresistant *Pseudomonas aeruginosa* strains from Germany and correlation with hypermutation. *Antimicrob. Agents Chemother.* 51, 4062–4070
23 Breidenstein, E.B. *et al.* (2011) *Pseudomonas aeruginosa*: all roads lead to resistance. *Trends Microbiol.* 19, 419–426
24 Fernández, L. *et al.* (2011) Creeping baselines and adaptive resistance to antibiotics. *Drug Resist. Updates* 14, 1–21
25 Taylor, P.K. *et al.* (2014) Antibiotic resistance in *Pseudomonas aeruginosa* biofilms: towards the development of novel anti-biofilm therapies. *J. Biotechnol.* 191, 121–130
26 Stover, C.K. *et al.* (2000) Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* 406, 959–964
27 Costerton, J.W. *et al.* (1995) Microbial biofilms. *Annu. Rev. Microbiol.* 49, 711–745
28 Friedman, L. *et al.* (2004) Two genetic loci produce distinct carbohydrate-rich structural components of the *Pseudomonas aeruginosa* biofilm matrix. *J. Bacteriol.* 186, 4457–4465
29 Ryder, C. *et al.* (2007) Role of polysaccharides in *Pseudomonas aeruginosa* biofilm development. *Curr. Opin. Microbiol.* 10, 644–648
30 Jenal, U. *et al.* (2006) Mechanisms of cyclic-di-GMP signaling in bacteria. *Annu. Rev. Genet.* 40, 385–407
31 Whiteley, M. *et al.* (2001) Gene expression in *Pseudomonas aeruginosa* biofilms. *Nature* 413, 860–864
32 Ghadaksaz, A. *et al.* (2015) The prevalence of some *Pseudomonas* virulence genes related to biofilm formation and alginate production among clinical isolates. *J. Appl. Biomed.* 13, 61–68
33 Drenkard, E. *et al.* (2003) Antimicrobial resistance of *Pseudomonas aeruginosa* biofilms. *Microbes Infect.* 5, 1213–1219
34 Sultana, S.T. *et al.* (2016) Eradication of *Pseudomonas aeruginosa* biofilms and persister cells using an electrochemical scaffold and enhanced antibiotic susceptibility. *Biofilms Microbiomes* 2, 2
35 Brooun, A. *et al.* (2000) A dose–response study of antibiotic resistance in *Pseudomonas aeruginosa* biofilms. *Antimicrob. Agents Chemother.* 44, 640–646
36 Webb, J.S. *et al.* (2004) Bacteriophage and phenotypic variation in *Pseudomonas aeruginosa* biofilm development. *J. Bacteriol.* 186, 8066–8073
37 Ghafoor, A. *et al.* (2011) Role of exopolysaccharides in *Pseudomonas aeruginosa* biofilm formation and architecture. *Appl. Environ. Microbiol.* 77, 5238–5246
38 Høiby, N. *et al.* (2010) *Pseudomonas aeruginosa* biofilms in cystic fibrosis. *Fut. Microbiol.* 5, 1663–1674
39 Garrett, E.S. *et al.* (1999) Negative control of flagellum synthesis in *Pseudomonas aeruginosa* is modulated by the alternative sigma factor AlgT (AlgU). *J. Bacteriol.* 181, 7401–7404
40 Schuster, M. *et al.* (2006) A network of networks: quorum sensing gene regulation in *Pseudomonas aeruginosa*. *Int. J. Med. Microbiol.* 296, 73–81
41 Lee, J. *et al.* (2015) The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. *Protein Cell* 6, 26–41
42 Hultqvist, L.D. *et al.* (2018) Imaging N-acyl homoserine lactone quorum sensing *in vivo*. *Methods Mol. Biol.* 692, 203–212
43 Laverty, G. *et al.* (2014) Biomolecular mechanisms of *Pseudomonas aeruginosa* and *Escherichia coli* biofilm formation. *Pathogens* 3, 596–632
44 Papenfort, K. *et al.* (2016) Quorum sensing signal-response systems in Gram-negative bacteria. *Nat. Rev. Microbiol.* 14, 576–588
45 Lee, J. *et al.* (2013) A cell-cell communication signal integrates quorum sensing and stress response. *Nat. Chem. Biol.* 9, 339–343
46 Filloux, A. *et al.* (2011) Protein secretion systems in *Pseudomonas aeruginosa*: an essay on diversity, evolution, and function. *Front. Microbiol.* 2, 155
47 Abby, S.S. *et al.* (2016) Identification of protein secretion systems in bacterial genomes. *Sci. Rep.* 6, 23080
48 Durand, E. *et al.* (2003) Type II protein secretion in *Pseudomonas aeruginosa*: the pseudopilus is a multifibrillar and adhesive structure. *J. Bacteriol.* 185, 2749–2758
49 Leone, P. *et al.* (2018) Type IX secretion system PorM and gliding machinery GldM form arches spanning the periplasmic space. *Nat. Commun.* 9, 429
50 Asfahl, K.L. *et al.* (2018) Additive effects of quorum sensing anti-activators on *Pseudomonas aeruginosa* virulence traits and transcriptome. *Front. Microbiol.* 8, 2654
51 Gholizadeh, P. *et al.* (2017) Suppressing the CRISPR/Cas adaptive immune system in bacterial infections. *Eur. J. Clin. Microbiol. Infect. Dis.* 36, 2043–2051
52 Høyland-Kroghsbo, N.M. *et al.* (2017) Quorum sensing controls the *Pseudomonas aeruginosa* CRISPR-Cas adaptive immune system. *Proc. Natl. Acad. Sci. U. S. A.* 114, 131–135
53 Heussler, G.E. *et al.* (2015) Clustered regularly interspaced short palindromic repeat-dependent, biofilm-specific death of *Pseudomonas aeruginosa* mediated by increased expression of phage-related genes. *MBio* 6 e00129–15
54 Chambers, J.R. *et al.* (2014) BrlR from *Pseudomonas aeruginosa* is a c-di-GMP-responsive transcription factor. *Mol. Microbiol.* 92, 471–487
55 Linares, J.F. *et al.* (2012) The global regulator Crc modulates metabolism, susceptibility to antibiotics and virulence in *Pseudomonas aeruginosa*. *Environ. Microbiol.* 12, 3196–3212
56 Hall, C.W. *et al.* (2017) Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiol. Rev.* 41, 276–301
57 Faure, L.M. *et al.* (2013) Phosphate starvation relayed by PhoB activates the expression of the *Pseudomonas aeruginosa* σvreI ECF factor and its target genes. *Microbiology* 159, 1315–1327
58 Skiada, A. *et al.* (2011) Adaptive resistance to cationic compounds in *Pseudomonas aeruginosa*. *Int. J. Antimicrob. Agents* 37, 187–193
59 Li, X.-Z. *et al.* (2009) Efflux-mediated drug resistance in bacteria: an update. *Drugs* 69, 1555–1623

60 Monday, S.R. *et al.* (1996) Alginate synthesis in *Pseudomonas aeruginosa*: the role of AlgL (alginate lyase) and AlgX. *J. Bacteriol.* 178, 625–632
61 Ma, L. *et al.* (2007) *Pseudomonas aeruginosa* Psl is a galactose- and mannose-rich exopolysaccharide. *J. Bacteriol.* 189, 8353–8356
62 Vasseur, P. *et al.* (2005) The pel genes of the *Pseudomonas aeruginosa* PAK strain are involved at early and late stages of biofilm formation. *Microbiology* 151, 985–997
63 Burrows, L.L. *et al.* (1997) *Pseudomonas aeruginosa* B-band O-antigen chain length is modulated by Wzz (Ro1). *J. Bacteriol.* 179, 1482–1489
64 Burrows, L.L. *et al.* (2002) Assembly pathways for biosynthesis of A-band and B-band lipopolysaccharide in *Pseudomonas aeruginosa*. In *Glycomicrobiology* (Doyle, R. J., ed.), pp. 127–143, Springer
65 Burrows, L.L. *et al.* (1996) Molecular characterization of the *Pseudomonas aeruginosa* serotype O5 (PAO1) B-band lipopolysaccharide gene cluster. *Mol. Microbiol.* 22, 481–495
66 Ernst, R.K. *et al.* (2006) The *Pseudomonas aeruginosa* lipid A deacylase: selection for expression and loss within the cystic fibrosis airway. *J. Bacteriol.* 188, 191–201
67 Cezairliyan, B. *et al.* (2013) Identification of *Pseudomonas aeruginosa* phenazines that kill *Caenorhabditis elegans*. *PLoS Pathog.* 9, e1003101
68 Bahl, C.D. *et al.* (2010) Crystal structure of the cystic fibrosis transmembrane conductance regulator inhibitory factor Cif reveals novel active-site features of an epoxide hydrolase virulence factor. *J. Bacteriol.* 192, 1785–1795
69 Ballok, A.E. *et al.* (2013) Pouring salt on a wound: *Pseudomonas aeruginosa* virulence factors alter Na+ and Cl− flux in the lung. *J. Bacteriol.* 195, 4013–4019
70 Liu, Y.C. *et al.* (2015) Modulation of host biology by *Pseudomonas aeruginosa* quorum sensing signal molecules: messengers or traitors. *Front. Microbiol.* 6, 1226
71 Glucksam-Galnoy, Y. *et al.* (2013) The bacterial quorum-sensing signal molecule N-3-oxo-dodecanoyl-L-homoserine lactone reciprocally modulates pro- and anti-inflammatory cytokines in activated macrophages. *J. Immunol.* 191, 337–344
72 Drees, S.L. *et al.* (2015) PqsE of *Pseudomonas aeruginosa* acts as pathway-specific thioesterase in the biosynthesis of alkylquinolone signaling molecules. *Chem. Biol.* 22, 611–618
73 Parsek, M.R. *et al.* (2000) Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc. Natl. Acad. Sci. U. S. A.* 97, 8789–8793
74 Amari, D.T. *et al.* (2013) The putative enoyl-coenzyme A hydratase DspI is required for production of the *Pseudomonas aeruginosa* biofilm dispersion autoinducer cis-2-decenoic acid. *J. Bacteriol.* 195, 4600–4610
75 Lamont, I.L. *et al.* (2003) Identification and characterization of novel pyoverdine synthesis genes in *Pseudomonas aeruginosa*. *Microbiology* 149, 833–842
76 Serino, L. *et al.* (1997) Biosynthesis of pyochelin and dihydroaeruginoic acid requires the iron-regulated pchDCBA operon in *Pseudomonas aeruginosa*. *J. Bacteriol.* 179, 248–257
77 Ochsner, U.A. *et al.* (2002) GeneChip® expression analysis of the iron starvation response in *Pseudomonas aeruginosa*: identification of novel pyoverdine biosynthesis genes. *Mol. Microbiol.* 45, 1277–1287
78 Davey, M.E. *et al.* (2003) Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.* 185, 1027–1036
79 Stehling, E.G. *et al.* (2008) Study of biological characteristics of *Pseudomonas aeruginosa* strains isolated from patients with cystic fibrosis and from patients with extra-pulmonary infections. *Braz. J. Infect. Dis.* 12, 86–88
80 Kessler, E. *et al.* (1998) Elastase and the LasA protease of *Pseudomonas aeruginosa* are secreted with their propeptides. *J. Biol. Chem.* 273, 30225–30231
81 Andrejko, M. *et al.* (2013) Three *Pseudomonas aeruginosa* strains with different protease profiles. *Acta Biochim. Pol.* 60, 83–90
82 Rocha, C.L. *et al.* (2003) Characterization of *Pseudomonas aeruginosa* exoenzyme S as a bifunctional enzyme in J774A.1 macrophages. *Infect. Immun.* 71, 5296–5305
83 Terada, L.S. *et al.* (1999) Pseudomonas aeruginosa hemolytic phospholipase C suppresses neutrophil respiratory burst activity. *Infect. Immun.* 67, 2371–2376
84 Cody, L. *et al.* (2009) *Pseudomonas aeruginosa* AlgR controls cyanide production in an AlgZ-dependent manner. *J. Bacteriol.* 191, 2993–3002
85 Chemani, C. *et al.* (2009) Role of LecA and LecB lectins in *Pseudomonas aeruginosa*-induced lung injury and effect of carbohydrate ligands. *Infect. Immun.* 77, 2065–2075
86 Iiyama, K. *et al.* (2007) Effect of superoxide dismutase gene inactivation on virulence of *Pseudomonas aeruginosa* PAO1 toward the silkworm, *Bombyx mori*. *Appl. Environ. Microbiol.* 73, 1569–1575
87 Elkins, J.G. *et al.* (1999) Protective role of catalase in *Pseudomonas aeruginosa* biofilm resistance to hydrogen peroxide. *Appl. Environ. Microbiol.* 65, 4594–4600
88 Lv, X. *et al.* (2017) Purification, characterization, and action mechanism of plantaricin DL3, a novel bacteriocin against *Pseudomonas aeruginosa* produced by *Lactobacillus plantarum* DL3 from Chinese Suan-Tsai. *Eur. Food Res. Technol.* 244, 323–331
89 Ding, X. *et al.* (2011) Screening for novel quorum-sensing inhibitors to interfere with the formation of *Pseudomonas aeruginosa* biofilm. *J. Med. Microbiol.* 60, 1827–1834
90 Cho, H.S. *et al.* (2013) Inhibition of *Pseudomonas aeruginosa* and *Escherichia coli* O157:H7 biofilm formation by plant metabolite ε-viniferin. *J. Agric. Food Chem.* 61, 7120–7126
91 Lidor, O. *et al.* (2015) Mechanistic analysis of a synthetic inhibitor of the *Pseudomonas aeruginosa* LasI quorum-sensing signal synthase. *Sci. Rep.* 5, 16569
92 Furiga, A. *et al.* (2015) Impairment of *Pseudomonas aeruginosa* biofilm resistance to antibiotics by combining the drugs with a new quorum-sensing inhibitor. *Antimicrob. Agents Chemother.* 60, 1676–1686
93 Ishida, T. *et al.* (2007) Inhibition of quorum sensing in *Pseudomonas aeruginosa* by N-acyl cyclopentylamides. *Appl. Environ. Microbiol.* 73, 3183–3188
94 Kohler, T. *et al.* (2010) Quorum sensing inhibition selects for virulence and cooperation in *Pseudomonas aeruginosa*. *PLoS Pathog.* 6, e1000883
95 Ilangovan, A. *et al.* (2013) Structural basis for native agonist and synthetic inhibitor recognition by the *Pseudomonas aeruginosa* quorum sensing regulator PqsR (MvfR). *PLoS Pathog.* 9, e100350895
96 O'Loughlin, C.T. *et al.* (2013) A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. *Proc. Natl. Acad. Sci. U. S. A.* 110, 17981–17986
97 Kim, H.S. *et al.* (2015) HD6-Gingerol reduces *Pseudomonas aeruginosa* biofilm formation and virulence via quorum sensing inhibition. *Sci. Rep.* 5, 8656
98 Kitao, T. *et al.* (2018) Molecular insights into function and competitive inhibition of *Pseudomonas aeruginosa* multiple virulence factor regulator. *MBio* 9, e02158–17
99 Morkunas, B. *et al.* (2012) Inhibition of the production of the *Pseudomonas aeruginosa* virulence factor pyocyanin in wild-type cells by quorum sensing autoinducer-mimics. *Org. Biomol. Chem.* 10, 8452–8464
100 Paczkowski, J.E. *et al.* (2017) Flavonoids suppress *Pseudomonas aeruginosa* virulence through allosteric inhibition of quorum-sensing receptors. *J. Biol. Chem.* 292, 4064–4076
101 Gunasekera, T.S. *et al.* (2017) Transcriptomic analyses elucidate adaptive differences of closely related strains of *Pseudomonas aeruginosa* in fuel. *Appl. Environ. Microbiol.* 83, e03249–16
102 Cathcart, G.R. *et al.* (2011) Novel inhibitors of the *Pseudomonas aeruginosa* virulence factor LasB: a potential therapeutic approach for the attenuation of virulence mechanisms in pseudomonal infection. *Antimicrob. Agents Chemother.* 55, 2670–2678
103 Chin, W. *et al.* (2018) A macromolecular approach to eradicate multidrug resistant bacterial infections while mitigating drug resistance onset. *Nat. Commun.* 9, 917
104 Kim, S. *et al.* (2016) PubChem substance and compound databases. *Nucleic Acids Res.* 44, D1202–D1213