Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics

Shugang Qin1, Wen Xiao1, Chuanmin Zhou2,3, Qinqin Pu3, Xin Deng4, Lefu Lan5, Haihu Liang4, Xiangrong Song6,8* and Min Wu6,8*

Pseudomonas aeruginosa (P. aeruginosa) is a Gram-negative opportunistic pathogen that infects patients with cystic fibrosis, burn wounds, immunodeficiency, chronic obstructive pulmonary disorder (COPD), cancer, and severe infection requiring ventilation, such as COVID-19. P. aeruginosa is also a widely-used model bacterium for all biological areas. In addition to continued, intense efforts in understanding bacterial pathogenesis of P. aeruginosa including virulence factors (LPS, quorum sensing, two-component systems, 6 type secretion systems, outer membrane vesicles (OMVs), CRISPR-Cas and their regulation), rapid progress has been made in further studying host-pathogen interaction, particularly host immune networks involving autophagy, inflammasome, non-coding RNAs, cGAS, etc. Furthermore, numerous technologic advances, such as bioinformatics, metabolomics, scRNA-seq, nanoparticles, drug screening, and phage therapy, have been used to improve our understanding of P. aeruginosa pathogenesis and host defense. Nevertheless, much remains to be uncovered about interactions between P. aeruginosa and host immune responses, including mechanisms of drug resistance by known or unannotated bacterial virulence factors as well as mammalian cell signaling pathways. The widespread use of antibiotics and the slow development of effective antimicrobials present daunting challenges and necessitate new theoretical and practical platforms to screen and develop mechanism-tested novel drugs to treat intractable infections, especially those caused by multi-drug resistance strains. Benefited from has advancing in research tools and technology, dissecting this pathogen’s feature has entered into molecular and mechanistic details as well as dynamic and holistic views. Herein, we comprehensively review the progress and discuss the current status of P. aeruginosa biophysical traits, behaviors, virulence factors, invasive regulators, and host defense patterns against its infection, which point out new directions for future investigation and add to the design of novel and/or alternative therapeutics to combat this clinically significant pathogen.

INTRODUCTION TO PSEUDOMONAS AERUGINOSA, A CRITICAL CLINICAL PATHOGEN AND MODEL MICROORGANISM

Pseudomonas aeruginosa is a multi-drug resistance (MDR) opportunistic pathogens, causing acute or chronic infection in immunocompromised individuals with chronic obstructive pulmonary disease (COPD), cystic fibrosis, cancer, traumas, burns, sepsis, and ventilator-associated pneumonia (VAP) including those caused by COVID-19.1–3 P. aeruginosa in biofilm states may survive in a hypoxic atmosphere or other extremely harsh environments.4 In addition, treatments of P. aeruginosa infection are extremely difficult due to its rapid mutations and adaptation to gain resistance to antibiotics.5 Furthermore, P. aeruginosa is also one of the top-listed pathogens causing hospital-acquired infections, which are widely found in medical devices (ventilation) because they tend to thrive on wet surfaces.2 Importantly, P. aeruginosa is one of the MDR ESKAPE pathogens, which stand for pathogens Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa, and Enterobacter. P. aeruginosa with arbapenem-resistance is listed among the “critical” group of pathogens by WHO, which urgently need novel antibiotics in the clinics.6

Epidemiological studies have shown that nearly 700,000 people died of the antibiotic resistance bacterial infections each year. P. aeruginosa that was isolated from European populations with a combined resistance was 12.9%.9 Hospital-acquired infection caused by P. aeruginosa continues to produce resistance to conventionally effective antibiotics becoming a main healthcare problem.10 The 2016 EPINE survey found that Escherichia coli and P. aeruginosa are the most common cause of hospital-acquired infections in Spain, P. aeruginosa accounting for 10.5% of clinically isolated bacteria infections.11 The 2011–2012 HCAIs report that P. aeruginosa caused almost
nosocomial 9% of infections, which is the fourth commonest pathogen of the European hospitals. Similarily, 7.1% of HCAI are caused by \textit{P. aeruginosa} in the United States. In addition, the 2016 European Center for Disease Prevention and Control (ECDC) epidemiological reported that \textit{P. aeruginosa} causes a variety of ICU-hospital-acquired infections, including pneumonia flares, urinary tract infections, and bloodstream infections. Furthermore, data from China Antimicrobial Surveillance Network (CHINET) identified 301,917 clinically isolated pathogenic strains and found \textit{P. aeruginosa} was the fourth nosocomial infections after \textit{Escherichia coli}, \textit{Klebsiella pneumoniae}, and \textit{Staphylococcus aureus}, accounting for 7.96%. Altogether, \textit{P. aeruginosa} is not a local, but a global major threat to human health.

The aforementioned statistics necessitate the identification drug targets and development of new treatments and effective vaccines for \textit{P. aeruginosa} to improve human health. However, both efforts have met huge difficulty due to the surging cases with MDR strains. This article broadly reviews the recent progress in \textit{P. aeruginosa} research towards the regulatory and functional mechanisms of virulence factors, gene expression regulators, secretion systems, quorum sensing, and antibiotic resistance, as well as host-pathogen interaction, new technologic advances, and therapeutic development (Fig. 1).

**VIRULENCE FACTORS**

\textit{P. aeruginosa} is able to adapt to the adverse environment in hosts by secreting a variety of virulence factors, which contribute to successful infection and causing disease. First, lipopolysaccharide (LPS) is an important surface structural component to protect the external leaflet and posion host cells and the endotoxicity of the lipid A in LPS enable tissue damage, attachment, and recognition by host receptors. In addition, drug resistance caused by the formation of biofilms is associated with the flagellum, pili, and other adhesins. Fourth, there are six types of secretion systems, including flagella (T6SS-associated), pili (T4SS), and multi-toxin components type 3 secretion system (T3SS), which function at colonisation of the host, adhesion, swimming, and swarming responding chemotactic signaling. Finally, exopolysaccharides, such as alginate, PsI and Pel, may help facilitate the formation of biofilms, while impairing bacterial clearance.

In terms of toxins, T3SS is a complex system and may severely impede host defense via injection of cytotoxins including ExoU, ExoT, ExoS and ExoY, which affect the intracellular environment, especially blocking phagocytosis and bacterial clearance. Exotoxin A (ETA) can inhibit host protein synthesis through ADP ribosylation.

*Fig. 1* Schema of \textit{P. aeruginosa} pathogenesis. \textit{P. aeruginosa} can be traced everywhere including hospital environments and cause serious infection of almost any organ. LPS induces TLR-4-dependent and -independent inflammatory responses in the lung after bacterial infection, epithelial cells secrete cytokines and chemokines, thereby recruiting and activating innate immune cells and adaptive immune cells. The recruitment of neutrophils is a sign of inflammatory response activation. Although the activation of neutrophils is critical for host defense, excessively activated immune cell infiltration will cause severe tissue damage and aggravate bacterial infections. Therefore, studying the balance between the virulence factors secreted by bacteria and corresponding host immunity is important for the treatment of infections.
activity.\textsuperscript{21,22} Pyocyanin is also toxic to hosts to cause disease severity, damage host tissue, and impair organs’ function.\textsuperscript{23} In addition, LasA and LasB elastases, alkaline protease (AprA) lipC lipases, phospholipase C, and esterase A enzymes comprise a large group of lytic enzymes that modulate other virulence factors.\textsuperscript{24} Moreover, rhamnolipids-mediated lung surfactant degrading and tight junction destroying can directly injure trachea or lung epithelial cells.\textsuperscript{25} Furthermore, siderophores (pyoverdine and pyochelin) as iron uptake systems help in bacterial survival in iron-depleted environment to augment virulence strength.\textsuperscript{26} Finally, antioxidant enzymes, such as catalases (KatA, KatB, and KatE), alkyl hydroperoxide reductases, and superoxide dismutases, neutralize activity of reactive oxygen species (ROS) in phagocyte environments to avoid clearance.\textsuperscript{27}

Virulence factors related to membranes

Lipopolysaccharide as a virulence factor widely interacting with hosts and also target for vaccines. LPS, an important classical structural component of the outer membrane (OM) of most Gram-negative bacteria, is a known potent agonist that elicits robust innate inflammatory immunity, its distal end may be capped with O antigen, a long polysaccharide that can range from a few to hundreds of sugars in length, which is critical for bacterial physiology and pathogenesis.\textsuperscript{28} At the early stage, scientists were interested in developing vaccines to prevent infection by focusing on LPS, which were later proven highly difficult due to the various serotypes and ineffectual outcomes.\textsuperscript{29,30}

Pathogen-associated molecular patterns (PAMPs), as small molecular motifs conserved in a class of microorganisms, can be sensed by toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) to activate innate immune responses, which effectively protect the host from infection.\textsuperscript{31} LPS, as the prototypical PAMP, can be recognized by multiple host receptors, including TLRs, PRRs, and nucleotide-binding oligomerization domain-like (NOD-like) receptors.\textsuperscript{32} The LPS-PRRs/NOD-like molecules activate inflammasome to produce proinflammatory cytokines.\textsuperscript{33,34} activating TNF-α and IL-1β, two of the most eminent inflammatory cytokines.\textsuperscript{35} Furthermore, LPS amongst five major Gram-negative bacteria have the ability to induce the production of NF-κB and proinflammatory IL-8 in a TLR4-dependent manner, suggesting that the pathogenesis of bacterial enhancement of chronic inflammatory diseases may be related to its serotype-specific LPS response.\textsuperscript{36}

Apparently, LPS exhibits a crucial role in regulating the interaction of bacteria with their host, is the main cause of tissue degeneration and chronic damage. LPS induces respiratory tract infections by regulating epithelial-mesenchymal transition (EMT)-mediated airway remodeling.\textsuperscript{37} The mutations of LPS can result in attenuated virulence.\textsuperscript{38,39} Caffeine alleviates the excessive inflammatory response caused by P. aeruginosa infection by inhibiting the activation of LPS-mediated TLR4/MyD88/NF-κB/miR-301b signaling pathway, and improves lung tissue injury.\textsuperscript{40} Notably, LPS mutations confer bacteria gain tolerance to phage infection,\textsuperscript{41} Taken together, in addition to the direct interaction with the host PRRs receptors, LPS may use its unique molecular features to adjust bacterial pathogenesis and damage host immune defense, ultimately benefiting the fitness and invasive strength.

OMVs are important part of virulence platforms. OMVs are bacterial components that can be released spontaneously to the environment during growth by many Gram-negative bacteria. Bacterial-derived OMVs have been characterized as a novel secretion mechanism that can deliver a variety of bacterial proteins and lipids into host cells without direct contact with host cells.\textsuperscript{42-44} OMVs can package and enrich a wide variety of proteins and nucleic acids, including lipoproteins, periplasmic proteins (E. coli cytolysin A, enterotoxigenic E. coli heat-labile enterotoxin, and Actinobacillus actinomycetemcomitans leukotoxin), plasmid containing chromosomal DNA fragments, phage DNA, virulence factors (LPS, alkaline phosphatase, phospholipase C, β-lactamase, and Cif et al.\textsuperscript{45,46} P. aeruginosa secretion of OMVs have been implicated in many virulence-associated behaviors, including the acquisition of drug resistance, the regulation of bacterial density and host immune escape.\textsuperscript{47-51} Mechanistically, P. aeruginosa secretes OMVs to deliver virulence factors and sRNAs into lung epithelial cells through the diffusion the mucus layer.\textsuperscript{44,47-52} Some studies also illustrate that OMVs could lead to an increased hydrophobicity of cell surface, resulting in enhanced ability to form biofilms.\textsuperscript{53} OMVs is controlled by quorum-sensing systems, which enable bacteria to colonize and immune escape.\textsuperscript{45,53} Interestingly, OMVs are naturally immunogenic and self-adjuration, making them have potential to be developed as antibacterial vaccine, such as OMV vaccine for Neisseria meningitidis.\textsuperscript{58-60} Therefore, OMVs are not only an important functional constituent, but also a potential biotechnological engineering carrier for vaccination or drug delivery. More details about virulence factors and their associated treatment strategies in P. aeruginosa are listed in Table 1 (45, 82–100).

SECRETION SYSTEMS, AN INTEGRAL PART OF VIRULENCE PLATFORMS AND MECHANISMS

Gram-negative pathogens cause various types of nosocomial infections, and secreted virulence factors often mediate their interactions with the host.\textsuperscript{61} Bacteria can modulate the host immune response through the secretion system for secreting virulence factors into host cells, which facilitates immune escape and enable bacterial colonization.\textsuperscript{62} Currently, 6 types of secretion systems (T1SS to T6SS) have been identified in P. aeruginosa. Based on the secretion routes of transport proteins, the secretion systems are divided into two major classes, one-step secretion system (T1SS, T3SS, T4SS, and T6SS) and two-step secretion system (T2SS and T5SS). The one-step secretion system directly secretes proteins from bacterial cytosol to the surface, while the two-step secretion system requires a brief periplasmic stay of the secreted proteins on the export way and then releases the proteins to outside environments of the bacterium (Fig. 2).

One-step secretion systems

T1SS. Two different T1SS types in P. aeruginosa elucidated, Apr secretion system and Has secretion system.\textsuperscript{63} The Apr secretion system consists of three major components: AprD (ATP-binding cassette transporter, ABC transporter), AprE (adaptor), AprF (outer membrane factor, OMF), and secretes two proteins: AprA (alkaline protease), and AprX (protein with unknown function).\textsuperscript{64} T1SS is found in a variety of bacteria (P. aeruginosa Salmonella enterica, Neisseria meningitidis, and E. coli).\textsuperscript{65} T1SS major transport proteins (such as proteases and lipases). The substrate protein containing a C-terminal uncleaved secretion signal were recognized by the ABC transporter, were directly transferred across bacterial inner and outer membranes in a one-step process.\textsuperscript{66} The Has secretion system is composed of HasD (ABC transporter), HasE (adaptor), HasF, and OMF.\textsuperscript{67} Has secretion system participates in iron regulation by secreting an extracellular haem-binding protein (hasAp).\textsuperscript{68} Thus far, data relating to T1SS is very limited and its function in pathogenesis and significance for bacterium physiology and fitness are largely unknown, requiring further elucidation in order to know whether it has potential important functions.

T3SS. The T3SS of P. aeruginosa, playing a key role in virulence like quorum sensing, was first discovered in 1996\textsuperscript{69} and is one of the most extensively-studied secreted toxins with increasing evidence for its important virulent effects.\textsuperscript{70} The T3SS regulon comprises five distinct operons, including the pscN to pscU, the exsD-pscB to pscL operons, the popN-pcr1-pcr2-pcr3-pcr4-pcrD-pcrR operon, the pcrGVH-popBD operon and finally the exsCEBA operon.\textsuperscript{71,72} The five distinct operons play important roles in the
**Table 1. The major pathogenesis factors of *P. aeruginosa* and therapeutics**

| Pathogenic factor        | Features and biological role                                                                 | Therapeutic intervention          | Vaccine availability |
|--------------------------|---------------------------------------------------------------------------------------------|-----------------------------------|---------------------|
| **Proteases**            | *P. aeruginosa* secreted proteases include elastase A, elastase B, large protease, protease IV, alkaline protease, Pseudomonas small protease, MucD, and *P. aeruginosa* aminopeptidase. They exhibit high proteolytic enzyme activity that damages host tissues by degrading proteins. | Protease inhibitors               | Preclinical         |
| **Toxins**               | *P. aeruginosa* produces a variety of extracellular toxins, including pigment, phytotoxins, hydrocyanic acid, phospholipase, protein convertase, enterotoxin, exotoxin, and mucus. These exotoxins can cause leukopenia, acidosis, liver necrosis, pulmonary edema, circulatory failure, renal tubular necrosis and bleeding, and many other serious damages. | Bacteriophages                    | Preclinical         |
| **LPS**                  | LPS is an integral component of cell envelope. It is the major virulence factor of *P. aeruginosa* and can be recognized by host pattern-recognition receptors to initiate inflammation and immunity response. | Antibody                          | Phase III           |
| **Pili and fimbriae**     | Pili and fimbriae are the major adherence factors. They contribute to the adherence and motility of *P. aeruginosa* in host. | Phages                            | None               |
| **Flagella**             | The main protein component of flagella is flagellin. Flagella provide motility and chemotaxis toward specific substrates and provide the ligand for clearance by phagocytic cells. | Bacteriophages                    | Phase III           |
| **Leukocidin (cytotoxin)**| They are secreted by the typical secretion system (e.g., ExoU secreted by Type III secretion system) and are the main cytotoxin targeting lymphocytes and neutrophils. | Natriuretic peptides              | None               |
| **Siderophores**         | There are two siderophores produced by *P. aeruginosa*: pyoverdine (formerly called fluorescein) and pyochelin. In addition to the iron needs, siderophores can support other virulence factors production by transferring iron, such as biofilms and toxic themselves. | Antibiotic-siderophore             | None               |
| **Urease**               | Urease enzyme is a virulence factor (limited extent) of *P. aeruginosa*. It can hydrolyze urea to produce ammonia and carbon dioxide (CO₂). It is associated with urinary tract infection. | None                              | None               |
| **Outer membrane proteins** | The outer membrane contains a large number of outer membrane proteins. These protein members are involved in the transportation of amino acids and peptides, the absorption of antibiotics, and the transportation of carbon sources. They are essential for bacterial adherence, virulence secretion, and host recognition. | Potential receptor for the internalization of host | Phase III |

**P. aeruginosa**: pathogenesis, virulence factors, antibiotic resistance, and treatment

Qin et al.

biogenesis and translocation mechanisms of type III secretions. Structurally, the T3SS, similar to a molecular syringe, comprises five components: the needle complex, the translocation apparatus, the regulatory proteins, the effector proteins, and the chaperones.75 T3SS secretes virulence effectors (ExoS, ExoT, ExoY, and ExoU) into the eukaryotic host cells to disrupt intracellular signaling and ultimately causing cell death.74–76 Many bacterial factors regulate T3SS genes of *P. aeruginosa*. The MgtC and OprF of PAO1 regulate T3SS and ExoS-induced host macrophage damage.77 The T3SS is positively regulated by PsrA,78 HigB,79 Vfr80 and DeaD81 but negatively regulated by MexT–82 AlgZR, GacAS/LadS/RetS,83–88 and MgtE.89 No only for *P. aeruginosa*, the T3SS is a highly important secretion mechanism for Gram-negative bacterial invasion factors, which may also facilitate the bacterial evasion from the host immune responses to establish negative bacterial invasion factors, which may also facilitate the bacterial evasion from the host immune responses to establish invasion, colonization, replication, and spread.

T6SS is a novel, important virulence machinery. In *P. aeruginosa*, T6SS is a newly identified powerful system with diverse and vital functions in virulence, bacterial interaction, and competition with the environmental microorganisms.90 Initially, the genome of *P. aeruginosa* was thought to constitute three gene clusters called Hcp Secretion Island (HSI) encoding T6SS components, which are later renamed H1-T6SS to H3-T6SS,91–92 with ~15–20 genes for each of them.93 In addition, the apparatus of T6SS, consisting of 13 core components, is divided into a baseplate-like structure, a sheathed inner tube assembled from the baseplate-like structure and a trans-membrane complex.94 The assembled T6SS appears to be an inverted phage tail, with the Hcp (hemolysin corregulated protein)–Vgr (valine-glycine repeat protein) complex forming the distal end of the cell-puncturing device.95 The sheath transits the effectors into targeted cells by a contraction-based mechanism.96 Furthermore, ClpV, an AAA+ family ATPase of T6SS, also provides the energy necessary to drive the secretory apparatus.97

Mechanically, the secretion process by T6SS needs other elements; for example, the H2-T6SS machinery to deliver the novel antibacterial toxin Tle3 requiring a cytoplasmic adaptor Tla3.98 The GacAS/Rsm regulates T6SS (H1-T6SS and H3-T6SS) by activating RsmY/Z to inhibit the binding activity of RsmA/RsmN to fha1/tssA.99 H3-T6SS secretes TseF to facilitate the import of the PQS-Fe³⁺ complex into cells by incorporating it into OMVs with *Pseudomonas* quinolone signal (PQS).100 Interestingly, it was found that the quorum sensing (QS) system plays an important role in the expression of this secretion system. In *P. aeruginosa*, QS differentially regulates three loci of encoding T6SS (HSI-I, HSI-II, and HSI-III) by LasR and MvfR.101 The QS systems regulator of Las and Rhl controls the expression of H2-T6SS in PAO1 strains102 and the QS regulator of MvfR directly modulates the expression of multiple proteins, including virulence factors and other regulators in PA14103 respectively.

The H1-T6SS-dependent substrates have a broad research foundation, while only little is known about functional roles of H2-T6SSs and H3-T6SSs due to the limited substrates available for research.104 With collaboration efforts, we characterize
H2-T6SS-dependent secretomes that are related to copper (Cu^{2+})-binding effector azurin (Azu). Furthermore, our studies reveal an Azu-interacting partner OprC, which is a Cu^{2+}-specific TonB-dependent outer membrane transporter and is also modulated by CueR. The Azu-OprC-mediated Cu^{2+} transport network may contribute to *P. aeruginosa* virulence. Our follow-up studies indeed illuminate that oprC dampens host response in cells and mice to *Pseudomonas* infection by potently enhancing Quorum-Sensing-associated virulence.

In addition, we recently describe a function for H2-T6SS of *P. aeruginosa* for specific delivery of AmpDh3 (a paralogous zinc protease) to the periplasm of a prey bacterium upon contact. AmpDh3 can hydrolyze peptidoglycan located on the cell wall of the prey bacterium to induce prey cell death, which serves as evolutionary advantage for *P. aeruginosa* in a competitive environment.

In spite of the relative short time since discovery of T6SS, the progress in understanding the potent virulence pathway is fascinating and fast-moving, which may define many unknown functions that can be attributed to T6SS' virulence and indicate new ways for treating patients who suffer the most severe disease and difficult to treat.

**T3SS and T6SS in cooperation for regulating host and bacterial responses.** T3SS and T6SS are indicated to regulate host and bacterial responses, including host cell apoptosis, inflammatory response, colonization, motility, biofilm formation, and bacterial competition/interaction (Fig. 3). Interaction regulation and inter-conversion of T6SS and T3SS may be especially helpful for coping with complex environmental pressures. The switch between T6SS and T3SS is directly regulated by the RNA-binding protein RtcB controlling colonization, establishment, and pathogenicity in *P. aeruginosa*. YbeY regulates T3SS and T6SS...
secretion systems and biofilm formation by controlling RetS. The function of T3SS is regulated by various regulators, including four main regulators genes (exsA, exsC, exsD, and exsE), which is involved in the transcription activation of the aforementioned classical effectors (exoS, exoT, exoU, and exoY). ExoS is a 48.3 kDa protein containing 453 amino acid length. It has been reported early that ExoS participates in host cell apoptosis via its GAP region or ADP-ribosyltransferase (ADPr) activity. Furthermore, the ExoS possesses ADPRT activity, which induces \textit{P. aeruginosa}-affected host cell apoptosis by targeting a variety of Ras proteins. ExoU is the longest \textit{P. aeruginosa} effector containing 687 amino acids (73.9 kDa). ExoU is the most acutely cytotoxic among the four effector proteins because it can induce rapid cell death and is considered to be the main driver of the cytotoxic phenotype. ExoU dysregulates the host’s innate inflammatory response by poisoning and killing immune cells, including macrophages, neutrophils, epithelial cells, and endothelial cells, allowing bacteria to persist, proliferate, and spread, and ultimately leading to sepsis, Alzheimer’s disease, acute respiratory distress syndrome, etc. Mechanistically, ExoU transiently represses Caspase 1 and NLRC4 inflammasome activation, inhibiting the release of IL-1β, IL-18, and proinflammatory DAMPs, and thereby suppressing the host immune response. In addition, ExoU can activate AP-1 transcription factors to increase IL-8 production and induce tissue-damaging inflammatory by JNK/MAPK pathway. ExoT containing 457 amino acids (48.5 kDa) has GAP and ADPRT activities, and can induce host cell apoptosis by targeting Crk proteins phosphorylation of p38 and JNK induces apoptosis, which subsequently interferes with integrin-mediated survival signaling via destroying the stability of focal adhesion sites. Notably, recent studies have shown that \textit{P. aeruginosa} ExoT induces G1 cell cycle arrest in melanoma cells, suggesting its potential for regulating the cell cycle. ExoY is a 378 amino acid protein with a molecular size of 41.7 kDa, detected in 80–100% of \textit{P. aeruginosa}. ExoY plays a direct role in immune escape by inhibiting TAK1 activation, which is a key factor in the TGF-inducible pathway that directly modulates immune responses, contributing to \textit{P. aeruginosa} survival and infection severity. In addition, ExoY regulates host inflammatory responses by delaying activation of NF-κB and caspase-1.

The T6SS components and their effectors are diverse and complex beyond bacterial-cell targeting. T6SS systems have
been detected in ~200 Gram-negative bacteria, including *P. aeruginosa*. To compete for survival in the living environment, H1-T6SS kills other bacteria by injecting Tse2 effector molecules into other target bacteria possessing antibacterial activity and providing advantages for *P. aeruginosa* growth. In addition, to protect itself from Tse2 toxins, *P. aeruginosa* also produces the antitoxin Tsi2. Similarly, H1-Tse1 and Tse3 are injected into the periplasm of other bacteria to hydrolyze peptidoglycan, which can be counteracted by periplasmic immune proteins Tse1 and Tse3. Tse4, Tse5, Tse6, and Tse7 that also show antibacterial activity are associated with homologous immunity. The phospholipase D enzymes PldA and PldB of Tle5 were injected to other bacteria by H2-T6SS and H3-T6SS to exert antibacterial activity. Further, VgrG2b is injected into epithelial cells by H2-T6SS, in which it targets the γ-tubulin ring complex component (γ-TuRC) and promotes the recruitment of PI3K at the apical membrane. Moreover, PldA/B targets the host PI3K (phosphoinositide 3-kinase)/Akt pathway to remodel the host by *P. aeruginosa*. Collectively, T6SS is a powerful antibacterial weapon that can be injected with many different effectors to compete with other bacteria and allow *P. aeruginosa* colonization and biofilm formation.

Two-step secretion systems

Different from the One-Step secretion, Two-step secretion requires a brief periplasmic phase of the secreted proteins on the export route before being exported to the outside of the cell through general export pathways, which plays an important role in the transport of periplasmic and outer membrane proteins.

T2SS. The function of T2SS is one of the less characterized secretion systems and is thought to secrete folded proteins from the periplasm. Two different pathways exist in T2SS: the general secretory (Sec) and twin-arginine translocation (Tat). The secreted proteins are first transited through the inner membrane, stays briefly in the periplasm and then secreted into the extracellular environment. The Sec pathway consists of a protein targeting component, a motor protein, and a membrane integrated conducting channel called SecYEG translocase, the secreted proteins with a SecB-specific signal sequence might be guided to the periplasm or the extracellular environment. The twin-arginine translocation (Tat) pathway of Gram-negative consists of TatA and TatB, which can decide whether the secreted is retained in the periplasm or translocated to the extracellular space with a twin-arginine motif. Functionally, T2SS participates in the secretion of guanylate cyclase ExoA, proteases LasA/B and multiple other factors and many of which have emerged as potential therapeutic targets.

T5SS. The T5SS of *P. aeruginosa* is composed of five subtypes (type Va to Ve) and exports the proteins across the inner membrane via the Sec pathway. The proteins of the V-type secretion system are often referred to as autotransporters (ATs). Typically, the T5SS consists of only one polypeptide chain with a β-barrel translocator domain in the membrane, and an extracellular passenger or effector region. Under the regulation of the Bam complex (β-barrel assembly mechanism) and TAM complex (translocation and assembly module), outer membrane proteins fold to form a β-barrel conformation and insert into the outer membrane. T5SS secretes a variety of proteins, including EstA, LepB, and LepA. EstA has esterase activity and is involved in rhamnolipid production and biofilm formation.

The type Vb secretion system comprises two distinct polypeptide chains encoded in one operon, therefore, it is also known as the dual-partner secretion system (TPSS) containing the passenger domain (TpsA) and the β domain (TpsB). TpsA has a TPS secretion motif and a functional/catalytic domain and the TpsB is a 16-chain OM-integrating β-barrel protein with two periplasmic POTRA (polypeptide transport-associated) domains. The Vc-type secretion system forms a highly intertwined trimeric structure and is therefore also known as a trimeric autotransporter adhesin (TAA). The C-terminal β-barrel domain of the Type Vd consists of 16 β-strands, similar to the β-barrel of TpsB proteins. The Ve-type ATs share obvious similarities with Va-type ATs. The main difference is that Ve-type ATs have an inverted domain order, with the β-barrel at the N-terminus and the passenger at the C-terminus. Despite these preliminary studies, there is much more to be learnt regarding T5SS for the physiology and virulence in *P. aeruginosa*.

T4SS. T4SS is a multisubunit cell-envelope-spanning structure that can transfer protein and nucleoprotein complexes across membranes, which is related to horizontal gene transfer-mediated antibacterial resistance, adaptation, evolution, and virulence. T4SS in bacteria is divided into the IVA-type secretion system represented by *Agrobacterium tumefaciens* VirB/VirD4 and the IVB-type secretion existed as *Legionella pneumophila* Dot/Icm system. Those distinct from the above are classified as “Other T4SS”, which contain genomic islands pKLC102 and PAPI in *P. aeruginosa* and are less characterized than other two types. *P. aeruginosa* T4SS comprises abundant major pilin subunits, PilA, and low abundance minor pilins FimU and PilVWX. Both major pilin and minor pilins are processed by the pre-pilin peptidase, PilD to exert function. T4SS assembly system is evolutionarily associated with T5SS contributing to the process of assembly and disassembly of pil. Minor pilins can impact assembly, retraction, extension, and adhesion.

Again, the chief function of T4SS is horizontal genetic transfer (HGT) between different microorganisms and potentially relating to pathogenesis. For the genetic island containing T4SS, there is a discrepancy: whether a conjugation mechanism exists but this is likely related to differences between strains. Nevertheless, there are relatively limited studies of this system compared to other secretion systems such as T3SS. We will further discuss the potential role of T4SS in host response context, initiating/activating inflammasome independent on nucleotide oligomerization domain (NOD)-like receptor (NLR) family CARD (C-terminal caspase recruitment domain) containing protein 4 (NLR4), in addition, the virulence factors and their associated secretion systems in *P. aeruginosa* were summarized in Table 2.

**VIRULENCE REGULATORY SYSTEMS**

The regulation of all these virulence factors is cell density-dependent through release of autoinducers of critical quorum sensing (QS) (e.g., Las, Rhl, Pqs, and lrp), a mass communication system. QS may help large population fitness by a hierarchical signal pattern to survive in fierce host environments and thrive, leading to persistent infection in individuals with cystic fibrosis, which cannot be completely cured even with tremendous progress in drug development, drastically improved medicare systems and living conditions. Hence, QS systems along with some other critical virulence factors, such as six types of secretion systems (of toxic molecules), two-component systems (TCSS), have become an intense interest in mechanistic understanding of this bacterium.
### Table 2. The virulence factors and their associated secretion systems in *P. aeruginosa*

| Secretion systems | Virulence factors | Features | Pathogenicity | Inhibition | Interaction/Regulation |
|-------------------|------------------|----------|---------------|------------|------------------------|
| **T1**            |                  |          |               |            |                        |
| Alkaline protease | Adherence and Colonization |          | Corneal infections | APRin         | Ca<sup>2+</sup>, Ca<sup>2+</sup> |
| HasAp             | Proliferation    |          | Scavenges heme, Metal complexes | hasR |                        |
| **T2**            |                  |          |               |            |                        |
| LasB (elastase)   | Biofilm formation |          | Antibiotic resistance, Ag-phendione, Cu(phendione) | IL-1β, LasA | 384, 395, 397, 404, 415, 431, 438 |
| LasA              | Biofilm formation and Colonization |          | Antibiotic resistance and Corneal infections | Satureja khuzistanica essential oil | 380, 381, 541, 548 |
| Phospholipase C (PlcH, PlcN) | Host inflammation regulation |          | Cell hemolysis and Neutrophil activity | Global regulator Anr | Ca<sup>2+</sup>, Pi, Zn<sup>2+</sup>, PlcR1, PlcR2, IL-1β, IL-8, 375, 386, 542, 545, 546 |
| PrPl protease     | Biofilm formation |          | Wound healing and Corneal virulence | – | Iron, PvdS, AprA, LasB |
| Lipases (LipA, LipC) | Motility, Biofilm formation and Rhamnolipid production |          | Enhanced PLC-induced 12-HETE and LTB4 generation | DsbC protein | 381, 390, 401, 411 |
| ToxA              | Colonization and Proliferation |          | Impairment of host defense, Inhibition of protein synthesis and Interference with cellular immune functions | Norepinephrine | 380, 384, 390, 401, 402 |
| LapA (via Hxc T2SS) | Biofilm formation and Localization adhesin |          | Antibiotic resistance | – | Lap5, c-di-GMP |
| Mep72             | –                |          | –             | –          | PdpD, PcrV, ExoS, FlID |
| **T3**            |                  |          |               |            |                        |
| ExoS              | Avoid phagocytosis and Kill the host cell |          | Inhibition of autophagy and mTOR, ROS Production and Induces apoptosis | Cinnamaldehyde and Canvacrol and honey | 375, 417 |
| ExoY              | Enhance acute pathogenicity and infection |          | Impairs endothelial cell proliferation and vascular repair | Aspirin | GSH, NF-xβ, AP-1, TAK1 |
| ExoT              | Inhibition of internalization by epithelial cells and macrophages |          | Inhibits lung epithelial wound repair | – | RhoA, Rac1, Cdc42 |
| ExoU              | Overexpression efflux pumps and AmpC β-lactamase genes |          | Antibiotic resistance and Augments neutrophil transepithelial migration | Arylsulfonamides | 389, 409, 426, 428 |
| ExE               | Negative regulator of type III secretion gene |          | –             | –          | T3SS |
| **T5**            |                  |          |               |            |                        |
| Esterase (EstA)   | Enhanced rhamnolipid production, Cell motility and Biofilm formation |          | –             | –          | ExsC, ExsD |
| CupB5             | Activates alginate overproduction |          | –             | –          | AlgU, Muca, TcpB4/LepB |
| Exoprotease (Lep) | Producing pyocyanin |          | –             | –          | Iron |
| **T6**            |                  |          |               |            |                        |
| Tse1 (amidase)    | Defense against other organisms |          | Lysis of cells | Tsi1 | Tsi1 |
| Tse2              | Defense against other organisms |          | Lysis of cells | Tsi2 | Tsi2 |
| Tse3 (muramidase) | Defense against other organisms |          | Lysis of cells | Tsi3 | Tsi3 |
| Other secretion systems |                  |          |               |            |                        |
| HCN               | –                |          | –             | –          | Glycine, AlgR, LasR and RrhR |
| Pyocyanin         | –                |          | Interferes with multiple cellular functions | Bactericid, 4-aminoypyridine | Phenylthiourea |
| Pyoverdine        | High-affinity iron uptake from transferrin and lactoferrin |          | Keratitis | – | PhdP tyrosinase |
| Rhamnolipids      | Biofilm formation |          | Potentiate aminoglycoside antibiotics | H<sub>2</sub>S | Oxygen, Polyhydroxyalkanoate |
| Alkyl quinolones  | Bacterial communication and quorum sensing |          | –             | –          | PqsR, LysR |
| N-acyl homoserine | Biofilm formation |          | –             | –          | Propolis |
| Lactones          | Biofilm formation |          | Accelerate host immunomodulation | Th1 and Th2 cytokines | 380, 401, 402 |
Quorum sensing is the most-studied systems for regulating gene expression and virulence

**Roles and acting mechanisms of QS.** QS describes a method that is widely utilized by bacteria for cell–cell mass communication. Both Gram-negative and -positive bacteria detect the local population density by sensing chemical signals and coordinate gene expression and group-behavioral benefits. Bacteria produce autoinducers or quorumone as diffusion signaling molecules and release into the environment for communication. Once the population reaching a threshold, the autoinducers activate their cognate receptors to directly or indirectly induce gene expression. Over the past two decades, QS has been extensively studied as a potential target for ‘antivirulence agents’, which may be harnessed to counteract bacterial virulence via a noncytotoxic mechanism as alternatives of traditional antibiotics.

Another essential function for QS in *P. aeruginosa* is to regulate the production of multiple virulence factors, such as extracellular proteases, iron chelators, efflux pump expression, biofilm development, swarming motility, and the response to host controlled gene expression during the stationary phase. VqsR, CdpR, which are required for PQS synthesis. AmpR activates QS-gene expression. Over the past two decades, QS has been activate their cognate receptors to directly or indirectly induce the expression of genes, has been the central point of research. For example, scientists have made significant efforts in understanding the interactions between all the four QS systems and also how environmental cues may affect gene expression and function of the QS. Two canonical N-acyl L-homoserine lactone (AHL) based (Las and Rhl) and two 2-alkyl-4 quinolones (AQ) based (Pqs and its precursor Hhq) signaling systems. These systems connect and coregulate each other. Rhl and Pqs were positively regulated by the Las system, while Rhl represses Pqs and Pqs augments Rhl. For example, in response to various nutritional and environmental stimuli, the regulatory relationship between Rhl and Pqs systems can change independently of Las.

The activation of QS genes generally requires a large number of regulatory factors to control receptor expression or function, and/or to coregulate some QS-controlled target genes since the QS systems are functional diverse, organizational complex, and consisting of a spectrum of key regulators (including rpaS, vqsR, rhlR, rsaL, etc.). RpoS indirectly plays a subtle role in activating lasR and rhlR expression and modulating ~40% QS-controlled gene expression during the stationary phase. VqsR controls the production of AHL signaling molecules and virulence factors by inhibiting the LuxR-type regulator QscR, which represses las expression to regulate the timing of QS activation. VqsM positively regulates the QS systems by controlling several relevant QS regulators ranging from QS to antibiotic resistance, and *P. aeruginosa* pathogenicity. MvaT and its homolog MvaU control the magnitude and timing of QS-dependent gene expression, which have a massive impact on all three QS systems by directly regulating lasR, lasI, rsaL, pphB, mvrR, algT/U, mvrR, and rpoS. RsaL binds to the lasI promoter and prevents LasR-mediated activation, regulates las signaling, and modulates the activity of PqSh and a recently identified regulator, CdpR, which are required for Pqs synthesis. AmpR activates QS-regulated genes to positively influence acute virulence, while negatively regulating biofilm formation. CdpR negatively modulates bacterial virulence by impacting the expression of pqsh, which is positively regulated by LasI and VqsM along with QscR and RsaL. We also recently found that BfrM/S and/or its variants modulate the rhl QS system in *P. aeruginosa*. Crc regulates rhl QS by promoting Hfq-mediated suppression of ion gene expression. More recently, our laboratory delineated that AnvM is a critical regulator of virulence in *P. aeruginosa* by directly interacting with the QS regulator MvrR and anaerobic regulator Anr. The aforementioned discoveries have highlighted the rapid progress in understanding diverse, heterogenous regulatory mechanisms of QS coordinated by seemingly a large number of unprecedented factors, which may finally characterize powerful, versatile regulatory proteins or systems to be applied to better control the notorious pathogen.

Several factors (such as QscR and QteE) have been identified to regulate the activation threshold of quorum-regulated genes, which control QS activation timing through additional homeostatic mechanisms. In addition to the QscR mentioned above, QteE also blocks QS-regulated genes’ expression by preventing LasR and RhlR accumulation and blocking Rhl-mediated signaling. Moreover, OsaL is found to interact with LasR.

Based on extensive studies to date, we may presume that QS may be one of the most important regulatory systems in *Pseudomonas* contributing substantially to bacterial physiology, adaptation, and pathogenesis. Although, various studies have tested the ideas of targeting QS for potential therapy of bacterial infection, the effect of using QS-associated approach for treatment is unsatisfactory. In particular, there were only limited reports of in vivo treatments targeting QS. Hence, it is necessary to further study the fundamental role of QS in bacterial pathogenesis and identify new anti-virulence targets and approaches that would help develop urgently needed medicines for treating refractory infection in clinics for QS bearing bacteria.

The two-component regulatory systems are critical gene regulators and virulence factors

The two-component systems (TCSs) are ubiquitous, complex signaling regulators that play vital roles in bacterial survival, metabolism, and virulence. As a versatile opportunistic pathogen, *P. aeruginosa* virulence network is tightly controlled by a growing number of TCSs. In general, a TCS pair of genes consists of a membrane-bound sensory histidine kinase (HK) and a cytoplasmic response regulator (RR). In *P. aeruginosa*, 64 HKs and 72 RRs have been identified. More than 50% of the TCSs and their corresponding HKs are linked to virulence and/or antibiotic resistance of *P. aeruginosa*. For instance, CzcCR/CzcS is implicated in carbapenem-resistance, KinB/AigB is involved in the alginate synthesis and virulence, and GacS/GacA is essential for pathogenicity. Remarkably, an attenuation in virulence behavior can be achieved by blocking TCS signaling. Goswami et al. reported that inhibition of HKs, especially Rlu-4 and 12, significantly reduced the production of virulence factors and toxins, and severely impacted the motility behavior of PA14.

Our recent studies discovered a new copper-responsive TCS called DsbRS in *P. aeruginosa*, in which DsbS (sensor of histidine kinase) and DsbR (cognate response regulator) modulate gene transcription for disulfide bond formation (Dsb). DsbS (phosphatase) targets DsbR to interfere with the transcription of Dsb genes and help the bacterium cope with copper stress. Intriguingly, transcription factors can also regulate the behaviors of bacteria to adapt host environments; for instance, imidazole-4-acetic acid (ImAA) and its receptor HikK are recently implicated in the response of *P. aeruginosa* to histamine. These findings help understand the communication between *P. aeruginosa* and hosts to adjust bacterial health. We have summarized TCSs and their roles in controlling the key virulence factors in *P. aeruginosa* (Table 3).

The number of TCSs related to the virulence of *P. aeruginosa* has recently grown considerably, which may be highlighted by the multi-kinase networks containing multiple sensor kinases through crosstalk (network) to impact virulence. The networks include: (1) GacS network governs the switch between acute and chronic infections; (2) Roc network and Rcs/Pvr network control cup

---

**Pseudomonas aeruginosa:** pathogenesis, virulence factors, antibiotic... Qin et al.
Small non-coding regulatory RNAs play roles in regulating gene expression and virulence
Small non-coding regulatory RNAs (sRNAs) range from 50 to 400 nucleotides in length. Since the discovery as part of a large group of transcompositional regulators in Escherichia coli in the 1960s, sRNAs are gradually recognized as key modulators to mount rapid responses when necessary and are encoded widespread in the bacterial genomes despite to different extent (the number of sRNAs in PAO1 is approximately twice higher than that in PA14). sRNAs play an essential role in bacterial pathogenicity and virulence mechanisms, such that participation in quorum-sensing regulation, ion metabolism, biofilm formation, stress responses, host cell invasion, and adaption to growth conditions. Hfq-dependent sRNAs are instrumental for modulating P. aeruginosa virulence and $rsmZ$ act as early responders to finely modulate bacterial cooperation under environmental stimuli to optimize population density.

In P. aeruginosa, sRNAs can regulate bacterial metabolism instantly and precisely to establish successful infection in the hosts. A total of 573 sRNAs were detected in PAO1 and 233 sRNAs in PA14, indicating their quantity variation in different strains. Although 126 sRNAs are found in common to both strains, sRNAs can evolve rapidly, and many sRNAs exhibit strain-specific or environmental-dependent expression. Interestingly, we recently revealed that sRNA PhrS may help generate CRISPR RNA (crRNA) for initiating bacterial immunity against bacteriophages, which is achieved through inhibiting Rho function on transcription-termination. Collectively, it would be intriguing to further understand the functions and underpinning mechanisms involving sRNA in this bacterium, which may identify novel pathway regulators and drug targets for controlling bacterial invasion.

### ACQUISITION OF ANTIBIOTIC RESISTANCE CONTRIBUTES TO BACTERIAL SURVIVAL AND THRIVING

The acquisition of drug resistance by P. aeruginosa depends primarily on multiple intrinsic and acquired antibiotic resistance mechanisms, including the biofilm-mediated formation of resistant and multi-drug-resistant persistent cells. Therefore, P. aeruginosa can quickly develop resistance to various antibiotics, including aminoglycosides, quinolones, and β-lactams.

In the course of long-term evolution, bacteria have developed a variety of ancient genetic resistance mechanisms that have significant genetic plasticity against harmful antibiotic molecules, enabling them to respond to various environmental threats, including possible harm (e.g., antibiotics, chemical compounds, and antimicrobial peptides) to their survival. The acquisition of antibiotic resistance with P. aeruginosa is quite diverse, and some primary mechanisms including outer membrane permeability, efflux systems, and antibiotic-inactivating enzymes will be addressed below (Fig. 4).
Outer membrane permeability
To treat *P. aeruginosa* infections, most antibiotics need to penetrate the cell membrane to reach the intracellular compartment to function. The outer membrane of *P. aeruginosa* can act as a specific hurdle inhibiting antibiotic penetration. The outer membrane of *P. aeruginosa* is chiefly composed of bilayer phospholipid molecules, LPS and porins embedded in phospholipids. The outer membrane is responsible for the specific and non-specific uptake of extracellular substances relying on different porin functions, including non-specific porins (OprF), specific porins (OprB, OprD, OprE, OprO, and OprP), gated porins (OprC and OprH), and efflux porins (OprM, OprN, and OprJ). *P. aeruginosa* manipulates different porins to limit antibiotic penetration and increase antibiotic resistance. OprF promotes the formation and attachment function of *P. aeruginosa* biofilm to protect the bacterium against antibiotics. Mutations of specific porins OprD involving conformational features cause carbapenem resistance, a serious challenge for medical treatment practices. Outer membrane protein H (OprH) of *P. aeruginosa* enhances the stability of the outer membrane through direct interaction with LPS to regulate antibiotic resistance. Efflux porins (OprM, OprN, and OprJ) contribute to the active efflux of several antibiotics, including tetracycline, norfloxacin, and β-lactam antibiotics. These findings demonstrate that the elegant effects and diverse mechanisms by which porins determine antibiotic resistance.

In a separate account, in recent years, OMVs secreted by *P. aeruginosa* are shown to be able to transport multiple virulence factors, like hemolytic phospholipase C, mRNA, DNA, β-lactamase, alkaline phosphatase, to the host cytoplasm, which may be a part of pathogenic mechanisms of antibiotic resistance. OMVs may be fused with the host plasma membrane through receptor-mediated endocytosis. While OMVs are detrimental to the host by delivering antibiotic resistance molecules or enzymes (β-lactamase), they have been harnessed as alternative delivery vehicles for transporting treatments or vaccines for various diseases including infection and cancer.

Efflux systems for pumping off drug resistance factors
The toxic compounds derived from metabolism or antimicrobials inside bacterial cells are harmful to bacterial survival, and require mechanisms to expel. The efflux pump is a main, conserved mechanism to remove antibiotics. The efflux pump may upregulate virulence genes (QS) for enhancing antibiotic resistance and maintaining bacterial homeostasis. Currently, five components are described in the efflux pump family: ABC
superfamily, major facilitator superfamily (MFS), and multidrug, toxic compound extrusion (MATE) family, resistance-nodulation-division (RND) family, and small multidrug resistance (SMR) family. Among all efflux pumps, the RND efflux pump family has the strongest correlation with antibiotic resistance. Twelve RND family efflux pumps are identified in P. aeruginosa: multidrug efflux AB-outermembrane porin M (MexAB-OprM), multidrug efflux CD-outermembrane porin J (MexCD-OprJ), multidrug efflux EF-outermembrane porin N (MexEF-OprN) and multidrug efflux XY-outermembrane porin M (MexXY-OprM).240,241 Quinolones MexEF-OprN are overproduced by the QS deficiency due to kynureneine extrusion.242 Furthermore, the resistance of P. aeruginosa to aminoglycosides, fluoroquinolones, and zwiterionic cephalosporins depends on the efflux function of MexXY-OprM.243 These findings suggest that despite some similarity in substrates, their affinity of efflux pumps may vary greatly, displaying different extent of anti-antimicrobial activities.

Multiple lines of evidence suggest that the chief mechanism for P. aeruginosa success in infection is highly related to its stubborn resistance to antibiotics or other therapeutics, which is regulated by the efflux pump. Hence, targeting this mechanism such as inhibiting the critical efflux pump—MexAB-OprM or enhancing the repressor—MexR, will likely reveal new strategies to overcome antibiotic resistance mechanisms in the bacterium and achieve the improved treatment efficacy.244

Antibiotic-inactivating enzymes that cleave enzymatic drugs

Antibiotics often contain chemical bonds (e.g., amides and esters), and bacteria can produce antibiotic-inactivating enzymes (hydro-lase) to degrade or alter antibiotics, leading to antibiotic resistance.245 P. aeruginosa is highly resistant to various antibiotics including penicillin, cephalosporin, and aztreonam by producing extended-spectrum β-lactamas (ESBLs).246,247 In addition, the bacterium is also resistant to the cefazolin-tazobactam combination therapy via ESBL GES-6.247 Again, the ESBL of P. aeruginosa is thought to be the most significant mechanism in terms of countering antibiotics, which would be a major target for designing and developing the most potent antimicrobial drugs.

From enzymatic angles, P. aeruginosa can modify the amino groups and glycosidic groups of aminoglycoside antibiotics to produce antibiotic resistance. The currently known aminoglycoside-modifying enzymes utilize three common mechanisms: aminoglycoside phosphotransferase (APH), aminoglycoside acetyltransferase (AAC), and aminoglycoside nucleotide transferase (ANT).249,250 These enzymes trigger the resistance of different antibiotics through various mechanisms, providing powerful resistant activities towards different types of antibiotics. APH can inactivate streptomycin by transferring the phosphate group to the 3'-hydroxyl group of aminoglycosides.241,245 AAC may cause gentamicin resistance by transferring the acetyl group to the amino group at the 3' and 6' positions of the aminoglycoside.243 ANT confers P. aeruginosa resistance to amikacin by transferring adenosine groups to the amino or hydroxyl groups of these aminoglycosides.254 The diverse enzyme list is growing (currently more than 50 enzymes), which helps in bacterial success in the anti-drug battle with humans.

Acquiring the antibiotic resistance throughout bacterial life cycle

Bacteria can acquire antibiotic resistance through mutations or horizontal gene transfer.255,256 Mutations of OprD in P. aeruginosa confers resistance to carbapenems248 and mutations of DNA gyrase (GyrA) causes resistance to quinolone antibiotics.257 Importantly, mutations in the β-lactamase gene ampC causes a significant increase in resistance to cephalosporins.258 There are already a host of enzymes in this bacterium may counter antibiotics while it continues to gain new resistance factors, which is debatably the biggest challenge for drug industry and scientific research. As bacteria can conveniently obtain antibiotic resistance genes from the same or different bacterial species through horizontal gene transfer, despite challenging targeting this mechanism may be a niche to search for better treatments.259 A typical example is that P. aeruginosa may obtain aminoglycoside and β-lactam resistance genes through horizontal gene transfer from the environment or other microbes at an unpredictable, fast pace.223,266 It may be highly difficult for scientists and clinicians to design new tools in impeding this natural robust mechanism in this bacterium.

Adaptive antibiotic resistance

When facing the diverse environmental stimuli, bacteria obtain adaptive resistance to increase the resistance to antibiotics through transient changes in gene and/or protein expression by a spectrum of approaches.261 In P. aeruginosa, the formation of biofilm is the most typical strategy to acquire adaptive antibiotic resistance.262,263 P. aeruginosa can produce biofilms to enhance pathogenicity. Meanwhile, P. aeruginosa infection can also cope with antibiotic treatment by forming persistent cells or persisters, thereby preventing the synthesis of antibiotic targets.262,263 Persistent molecules from the persisters can maintain vitality and refill biofilms; once antibiotics are not present, bacteria will resume growth and cause chronic infections.264 Altogether, P. aeruginosa can become exceedingly resistant to antibiotics through myriad of mechanisms, and currently we probably only know a tip of iceberg regarding these bacterial behaviors. It necessitates accelerated research efforts to fully understand the detailed mechanisms by which bacteria constantly grow antibiotic resistance, providing insight into the design and development of innovative and efficacious drugs to overcome drug resistance.

HIGHLY COMPLEX AND MULTIPLE MECHANISMS IN HOST IMMUNE RESPONSES TO THE BACTERIUM

The opportunistic pathogen P. aeruginosa exists almost everywhere and in any environmental conditions. In immunosuppressed people, there is extreme susceptibility to P. aeruginosa infection, developing either acute or chronic phenotypes. As the first line of host defense systems, the innate immune system plays a vital role in battling with P. aeruginosa via multiple mechanisms, such as phagocytosis and inflammatory responses. Several types of host systems, such as pattern recognition receptors (PRRs), plasma membrane signals, intracellular enzymes, and secreted cytokines/chemokines participate in inflammatory response against the bacterial infections. Although a well-balanced inflammatory response is required for restraining P. aeruginosa invasion, overzealous inflammation is associated with rapid disease progression, tissue injury, and even death. Some host molecules including cytosolic protein annexin A2 (AnxA2), autophagy-related protein 7 (ATG7), NLRC4, as well as non-coding RNAs (IncRNAs and microRNAs), are also implicated in P. aeruginosa-induced inflammation and/or other aspects of host defense mechanisms,265,266 and understanding of the mechanisms of inflammatory response is just beginning to be unfolded.

Toll-like receptors (TLRs) instantaneously recognize invasive pathogens

TLRs are highly conserved transmembrane PRRs, containing leucine-rich repeats and Toll/interleukin-1 receptor (TIR) homolog domains, which rapidly recognize invading microorganisms and elicit innate immunity and inflammatory response upon bacterial invasion.269 TLRs play vital roles in initiating innate immunity for eradicating invading pathogens.269 Upon the sensing of
pathogen-associated molecular patterns (PAMPs), TLRs activate NF-κB and AP-1 to mediate inflammatory response signal pathways. Correspondingly, TLRs are capable of recognizing different pathogen-associated molecular patterns found in *P. aeruginosa*. LPS is a major component of the outer membrane of *P. aeruginosa*, responsible for maintaining bacterial membrane structure and initiating immune response as a major antigenic factor. Research shows that a large amount of hex-acylated lipid A from LPS is isolated from infected patients, which is a strong TLR4 agonist, capable of activating TLR4-dependent inflammatory response. In addition, several TLR4 co-receptors MD-2 and CD14 are indicated necessary for TLR4 recognition of LPS and TLR4 activation. Airway epithelial cells and macrophages, both expressed TLR4 in the cell membrane, serve as the first line of host defense for the initial contact to *P. aeruginosa*. As TLR4 is the essential trigger of host immunity against *P. aeruginosa* infection, its deficient mice show higher bacterial burdens and severe lung injury under infection. Nevertheless, the TLR4 pathway does not function alone and is very complex, which may be impacted or coregulated by a number of signaling systems including AnxA2, autophagy, cGAS, cGAMP, ion channel proteins, DNA repair proteins, miRNAs, and IncRNA, to name a few.

Apart from TLR4, TLR2 has also been reported as an LPS recognizer, which is capable of recognizing *P. aeruginosa*-derived lipoproteins and T3SS effector ExoS. In addition, TLR5 is involved in detecting exogenous PAMPs like TLRs, which are also essential for *P. aeruginosa* infection. TLR5 is playing out by TLRs using a comprehensive approach or systems biology in an unbiased manner in animals as well as human subjects, which may provide insight into the disease pathogenesis and suggesting new therapeutic development. Nucleic acid sensor cGAS is a pivotal for eliciting immune reaction against *P. aeruginosa*. cGAS is a recently discovered nucleic acid sensor that is initially identified to recognize viruses. Typically, activation of cGAS contributes to the induction of inflammation as a means against viral or intracellular bacterial infection. However, the latest research shows that cGAS downstream effector STING may also play an anti-inflammatory role under extracellular bacterial *P. aeruginosa* infection by inhibiting NF-κB activity. More recently, we have found that cGAS may be involved in an uncoupled protein response (UPR) during *P. aeruginosa* infection. Mechanistically, our studies uncover cGAS as a novel nucleic acids’ sensor to initiate immune responses against *P. aeruginosa* infection through a canonical pathway involving STING and IRF-1, suggesting that cGAS pathways may be a critical target for control of this bacterium. It is a new and important function for cGAS since previous reports were primarily focused on viral sensing and intracellular bacterial sensing.

**Inflammasomes drive inflammation and pyroptosis**

The inflammasome is a multiprotein complex, which is attributed to the production and release of inflammatory cytokines, IL-1β and IL-18. Recent work reveals that inflammasome is involved in pyroptosis dependent on the cleavage of Gasdermin D, which contributed to the formation of plasma membrane pores, and in turn promoting the release of inflammatory cytokines and pyroptosis. Typically, inflammasome consists of cytosolic PRRs, ASC (an apoptosis-associated speck-like protein containing a CARD), and caspase 1. Inflammasome PRRs are responsible for detecting exogenous PAMPs like TLRs, which are also essential for monitoring *P. aeruginosa* invasion and activate host inflammatory response for promoting the clearance of *P. aeruginosa*. Remarkably, TLRs are involved in the priming of inflammasome activation by promoting the transcription of inflammasome-related genes. NLR4 and AIM2 (absent in melanoma 2) are well characterized among numerous inflammasome PRRs linked to infection of *P. aeruginosa*.

**NLR4 inflammasome is shown capable of recognizing needle and inner rod (Prgl) T3SS proteins, independently of T3SS exotoxins, or intracellular flagellin utilizing different murine NAIP as adaptors.** Unexpectedly, only one factor, human NAIP (hNAIP), has been found, homologous to murine NAIP5, responsible for sensing *P. aeruginosa* T3SS inner-rod protein PscI and needle protein PscF. Apart from T3SS, the T4SS pilin is also capable of activating inflammasome independent of NLR4 and ASC. *P. aeruginosa*-induced mitochondrial dysfunction also promotes the assembly and activation of NLR4 via T3SS. Mitochondrial ROS and release of mitochondrial DNA are key to NLR4 activation under *P. aeruginosa* infection, which is also dependent on autophagy. Removal of damaged mitochondria blocks the activation of NLR4. However, AIM2 appears to be dispensable for recognizing and promoting *P. aeruginosa* infection-induced inflammation in most cases.

Although several *P. aeruginosa* components are implicated in activating inflammasome-related immune defense, little is known about how the bacterium evades immune response after inflammasome activation. Recent research broadens our knowledge that *P. aeruginosa* takes advantage of bacterial QS-dependent secretant, including proteases, pyocyanin, and 3-oxo-C12-HSL, to inhibit the activation of NLR4 via NLR family, pyrin domain containing 3 (NLRP3) inflammasomes. A further study supports that pyocyanin specifically inhibits activation of the NLRP3 inflammasome (but not NLR4 and AIM2) through induced excessive oxidation. Contrary to the positive role of oxidation in NLRP3 activation, hence, the potential role of oxidation in *P. aeruginosa* infection and inflammasome activation requires further study. In addition, K⁺ efflux is necessary for the activation of inflammasome against *P. aeruginosa* infection.

**To date, the role and underlying mechanisms of the inflammasome in host defense against *P. aeruginosa* remain largely unclear and sometimes paradoxical: some researches show that inflammasome activation enhances host defense to clear *P. aeruginosa*, whereas others present opposite results that inflammasome activation triggered severe host tissue damage, which may impact disease progression and mortality. One explanation for such a mixed response is that infection may involve different pathways dependent on the bacterial strains, model systems, environments, and experimental conditions. Another caveat is that most experiments have not been performed at holistic and/or spatiotemporal levels to evaluate the dynamics, rather a single cell type, specific location, and one or two timepoints. Therefore, enhanced or advanced approaches are needed to further clarify the role and underlying regulatory mechanisms of inflammasome activation in *P. aeruginosa* infection.**
systems (types I, III, and IV systems) are dependent on a single CRISPR-Cas effector protein. CRISPR-Cas I-C, type I-E, and type I-F systems have been found in many clinically isolated P. aeruginosa. It is known that CRISPR-Cas systems provide adaptive immunity against the invasion of bacteriophages or plasmids. However, the role of CRISPR-Cas systems is far beyond the adaptive immunity of bacteria based on the current reports. Our research showed that type I CRISPR-Cas targeted the QS regulator LasR to inhibit toll-like receptor 4 recognition, thereby evading mammalian host immunity, suggesting that the CRISPR system is linked to host immunity modulation by targeting their own genes, potentially evading host defense mechanisms. Type I CRISPR-Cas systems may elicit inflammasome activation in mammalian hosts by regulating autophagy in P. aeruginosa. To ensure maximum CRISPR-Cas function, P. aeruginosa utilized quorum sensing to activate the expression of cas genes to promote CRISPR adaptation when bacteria are at high risk of phage invasion. The adaptability and virulence of P. aeruginosa can be regulated by CRISPR-Cas adaptive immunity based on the biological complexity of microbial communities in natural environments. The CRISPR-Cas system actively maintains the virulence of P. aeruginosa by limiting virus-like accessory genomic elements. Increased expression of phage-related genes initiates CRISPR-mediated biofilm-specific death of P. aeruginosa. CRISPR-Cas systems may help mediate antibiotic resistance to multiple membrane irritants, including enhancing the integrity of the envelope in pathogen Francisella novicida. Consequently, various corresponding anti-CRISPR (Acr) proteins have evolved by phages to inhibit bacterial CRISPR systems. Our previous research identified a series of type VI-A anti-CRISPRs (acrVIA1-7) genes that block the activities of Type VI-A CRISPR-Cas13a system, and designed Type III CRISPR endonuclease antivirals for coronaviruses (TEAR-CoV) as an experimental therapeutic to combat SARS-CoV-2 infection, suggesting that exploiting the mechanism of CRISPR-mediated adaptive immunity has great potential for treating bacterial and viral infections. However, it is not clear whether CRISPR-Cas systems can regulate antibiotic resistance in P. aeruginosa, which may be studied in future (Fig. 5).

DEVELOPMENT OF NOVEL THERAPEUTICS FOR P. AERUGINOSA

P. aeruginosa have strong ability to develop natural and acquired drug resistance through various mechanisms, including the production of antibiotic inactivating enzymes or antibiotic modifying enzymes, inhibiting the penetration of the drug through the cell wall, changing the target of antibacterial action, and limiting the drug to reach its target and adaptation to the adverse environment. Due to the increasing difficulty in treating antibiotic resistance strain infection, the development of the anti-P. aeruginosa therapy depends on targeting the resistance mechanism. Research on the resistance mechanism of P. aeruginosa has been an urgent topic for decades since antibiotic resistance has escalated exceedingly. Even with the intense interest, development of new antibiotics and other therapeutic strategies for P. aeruginosa infections is at a painstakingly slow pace due to the variability and complexity of drug resistance, as well as the lack of a deep understanding of the pathogenic mechanisms for P. aeruginosa. Designing effective therapeutic approaches (including phage therapy, immunotherapy, gene editing therapy, antimicrobial peptides, and vaccine therapies) to counteracting P. aeruginosa invasion has been an increasing urgency, requiring consorted efforts (Fig. 6).
New antibiotic formulations and compounds

With an alarming rise in pathogens with resistance to existing drugs, a number of new antibiotics have recently entered the antibiotic development pipeline; however, the hope for patients and clinicians is rapidly dwindling once a new antibiotic resistance strain emerges. Hence, we should invest unconventionally research efforts in searching for new treatments of MDR bacteria.

Recently developed antimicrobials are discussed below.

The biological activity of substituted guanidines was known in the mid-1930s when a series of guanidines and metformin compounds were found to possess bactericidal and disinfectant properties. Subsequently, many guanidine derivatives were studied for therapeutic purposes. Chin et al., recently reported a polyguanidine polycarbonates with strong antimicrobial activities through a distinctive mechanism that does not intensify drug resistance in multi-drug resistance (MDR) superbugs including methicillin-resistant S. aureus, P. aeruginosa, A. baumannii, K. pneumoniae, suggesting that the polyguanidine compounds may be potential antibacterial candidates.

In addition, novel antimicrobial peptides are promising to become the next generation of antimicrobials. The sequences of amino acids with antibiotic nature can be found in insects, soil bacteria, amphibians, plants, and even mammals. With the diversity of species and antimicrobial mechanisms, peptides render a niche as an alternative treatment for the MDR bacteria.

A variety of new antibiotic formulations for treating P. aeruginosa infection have been tested in the clinic despite mostly at preliminary stages. Plazomicin is a novel semi-synthetic parenteral...
aminoglycoside that inhibits bacterial protein synthesis, and is shown to inhibit P. aeruginosa growth.\textsuperscript{137} Plazomicin is assessed in two Phase III randomized controlled trials, with an EPIC trial compared with meropenem in Complicated Urinary Tract Infection (cUTI). Plazomicin demonstrated a composite cure 81.7% (P) vs. 70.1% (M), a difference of 11.6%.\textsuperscript{137} Similarly, another trial CARE with plazomicin-based or colistin-based combinations to treat infection by carbapenem-resistant Enterobacteriaceae (CRE) shows that therapy with plazomicin-based combinations reduced mortality and complications vs. colistin-based combinations (23.5% vs 50%). This latter (CARE) trial seems more effective than the former (Plazomicin) and is achieved by enhanced sustained microbiological eradication.

Eravacycline is a novel fluoroquione that is evaluated for antimicrobial activity against anaerobic Gram-negative and Gram-positive bacteria. Eravacycline demonstrates potent broad-spectrum activity against 90% of bacterial isolates having a concentration range of ≤0.008 to 2 μg/ml.\textsuperscript{338} Eravacycline is more effective when an expression of tetracycline-specific efflux and ribosomal protection mechanisms is present. Eravacycline is effective against multi-drug-resistant bacteria towards other antibiotic classes. Eravacycline has shown broad-spectrum anti-bacterial activity when put unique chemical modifications at C-9 and C-7 of the tetracycline core.\textsuperscript{338}

Baxdela, also known as delafloxacin meglumine, is a broad-spectrum anionic fluoroquinolone and has distinct chemical structures that increase potency in acidic environments. Delafloxacin is known for inhibiting DNA replication and repairs targeting DNA gyrase and topoisomerase IV. Currently, Baxdela is under a Phase III clinical trial for therapy of community-acquired pneumonia.\textsuperscript{308} Zidebactam (WCK 5222) is a dual mechanism antibiotic in a development phase, which is involved in binding Gram-negative PBP2 to exert β-lactamase inhibition. As an active antibiotic against Enterobacteriaceae producing CTX-M-15, Zidebactam has some effects against Enterobacteriaeae and P. aeruginosa.\textsuperscript{339} Although we only reviewed a small portion of ongoing development of new therapeutics for P. aeruginosa with some hope, development of effective therapeutics to counter the growing drug resistance is still challenging.

Nanoparticles may be useful for delivering drugs or vaccines. Nanoparticles have been tested in a number of therapeutic applications, such as drug delivery, gene delivery, and vaccine delivery in addition to direct killing of bacteria by its membrane piercing ability and induction of ROS. Since the CRISPR/Cas system can target to self-genomes within a bacterium, delivery of CRISPR-Cas targeting a vital gene (for survival) by nanoparticles may directly kill the bacterium. Use of nanoparticles for delivering vaccines can also be achieved but preparation of optimal particles that can readily control protein loading and folding remains challenging.\textsuperscript{430} We will focus on the studies of vaccines and nanoparticles. There are several types of nanoparticles: nanoantibiotics liposomes, polymers, hydrogels, metallic particles, magnetic structures, carbon-based materials, and mesoporous with silica and each has its advantages and disadvantages. Hence, a multivalent nanoparticle strategy is considered a superior means and is used in vaccine delivery with P. aeruginosa, specifically to target T6SS. T6SS can divide into two distinct forms: phage tail-like structure and transmembrane complex, which can be exploited to create multivalent nanoparticle delivery of vaccine antigens. For the delivery of T6SS structural device, many proteins in a single nanoparticle may be delivered simultaneously.

The drawback of using nanoparticle structures is the likelihood that host immune responses may be activated against the delivery system, which can prohibit the repeated delivery.\textsuperscript{430} Future experiments need to be conducted to control antigen exposure to generate a uniform particle. The advantages of T6SS delivery system can be adopted for other gene targets with different nanoparticles. The advancement of nanotechnology has allowed a variety of nanostructures as a strategy to minimize the undesirable characteristic and natural and synthetic AMPs. Reports have indicated that in nanostructures, peptides present lower cytotoxicity and better efficiency of desired targets. Hence, nanostructures may help produce biomolecules and implementation of vaccines and drugs against extracellular degradation and target treatment. Using two approaches for encapsulating AMPs, nanotechnology can use an indirect approach where a passive delivery occurs involving a conventional nano-delivery system. Direct delivery is involved in an active target for modifying the surface chemistry of the nano-carrier with ligands. When comparing the two approaches, both have their rpos and cons, using the passive system involving the encapsulated peptide results in the best fitting solution.\textsuperscript{364}

As biofilms are a major cause of drug resistance, nanoparticles targeting biofilms are also a promising strategy. Zhang et al. found that magnetite hybrid nanocomplexes can penetrate and disrupt bacterial biofilms by breaking through the biofilm matrix barrier.\textsuperscript{41} Others also demonstrate that silver nanoparticles (metallic particles) combined with polymyxin B can target P. aeruginosa biofilms with significant effects.\textsuperscript{342} Biofilms are also an important factor to aggravate cystic fibrosis disease. Hence, exotoxin A (ETA) is encapsulated into poly-lactic-co-glycolic acid (PLGA) nanoparticles, forming the ETA-PLGA nanoparticles, which may function as a continuous immunogen to trigger cellular immunity.\textsuperscript{343} Therefore, these nanoparticles may be one of the potential vaccine candidates to penetrate biofilms. To date, many nanoparticles-mediated compounds are in clinical development and several of them have been approved for human therapy, especially cancer therapy, hepatic fibrosis, virus infection, fungal infections, hypercholesterolemia, and pneumonia.\textsuperscript{344–346} In particular, the recently approved lipid nanoparticle (LNP) mRNA vaccines for COVID-19 was an acclaimed success in nanoparticle research for contribution to novel vaccines to combat diseases.\textsuperscript{347}

Phage therapy is a reviving alternative therapy for severe and incurable cases. Due to the lack of effective therapy for drug-resistant superbugs, phage therapy has been a viable alternative for bacterial treatment, especially for patients who have been non-responsive to conventional antibiotics. There are two types of bacteriophages, lytic and temperate, only lytic phages are utilized for clinical therapy. Lytic phages adhere to the host cell surface and inject DNA into host cells. Proliferated phages release and migrate towards infection sites through targeting specific bacteria. For P. aeruginosa, flagella vaccines have been used together with phage or other therapies and showed the remarkable therapeutic results. However, P. aeruginosa displays heterogeneity and varying clinical outcomes with acute infections in later phases, where bacteria start losing their flagella and pili. A vaccine based on flagella will direct toward motile bacteria instead of established/colonized biofilms-forming strains.\textsuperscript{349} Advanced techniques have been developed for formulating phage cocktails to improve efficacy in vitro. For example, Forti et al. made a cocktail with six phages: E215, E217, PAK_P1, PYO2, DEV, and PAK_P4 and showed some effects against clinical P. aeruginosa strains.\textsuperscript{348} A number of studies showed that phage cocktails possess enhanced efficiency in killing P. aeruginosa compared to single phage therapy.\textsuperscript{350,351} As strains from patients are distinct, personalized phages combined with antibiotics have also been applied for effective and safe therapy in clinics. An excellent such application was reported by Forti et al., who constructed phage cocktails based on the genomic information and host range.\textsuperscript{349} Clinical trials of phage therapy have shown viable and sustained efficacy in therapy. Marked reduction in bacterial colonies with a single dose of phage therapy has been reported in small studies.\textsuperscript{352} Previous studies have primarily been on patients with burns. Phages were applied on the wounds, but
the minimal effect was observed.\textsuperscript{353} With \textit{P. aeruginosa} being a ubiquitous pathogen that infects lungs and many other organs, further research will investigate how phage therapy would help control chronic CF or COPD diseases. Phage neutralizing antibodies that are induced by the body's immune system and the safety concern about exotoxins during phage preparations are among the biggest challenge of phage therapy.

Another limitation is that bacteria can become resistant rapidly to phage therapy due in part to CRISPR-Cas systems, which are a bacterial adaptive immunity system that can degrade invading DNA in a high specificity. Research has been conducted with a CRISPR-Cas system that targets endogenous genes to alter host defense.\textsuperscript{324} To conquer the bacterial immune response for promoting phage replication, phages should gain access to do the job by evolving an anti-CRISPR system (Acrs). With this mechanism, future goals are to use natural anti-CRISPR or synthetic proteins that can counteract the CRISPR systems.\textsuperscript{319} A protein called AcrIF was shown to be a potent inhibitor of the CRISPR system in \textit{P. aeruginosa}. This protein is locked with the Cys complex of CRISPR and diminished the phages' ability to bind to DNA.\textsuperscript{354} Further research may be conducted using the same mechanism for bacteria to become resistant to CRISPR. Creating a synthetic phage with desired characteristics and genomic content would be a creative approach.\textsuperscript{355} Recently, mutations in phage host-range-determining regions (HRDRs) to cope with resistance mutation of bacteria have been implemented as an effective method to prevent \textit{Escherichia coli} infection.\textsuperscript{41} Engineered bacteriophages BPS\textsubscript{Δ33}HIF\_HRM10 and D29\_HRMGD40 cocktail cured a cystic fibrosis patient with a disseminated drug-resistant \textit{Mycobacterium abscessus}, which shows that the therapeutics of engineered phages to break the evolutionary constraints, holding great potential to create the next-generation of antibacterial antimicrobials.\textsuperscript{356,357} In spite of recent progress, the strategy for phage therapy is highly difficult. Through creative design, basic research and clinical testing, phage therapy can be rejuvenated against bacterial infections. Hence, it is promising to combine the phage therapy and anti-CRISPR approach for development of clinically feasible antibacterials.\textsuperscript{353}

Gene delivery has made notable progress

Genome editing and nucleic acid-based antibiotics, such as single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), plasmid DNA, and ssRNA\textsuperscript{358} have emerged as the new types of antimicrobials. For gene editing, there is no doubt that editing with CRISPR systems is of high promise. Since scientists first used Cas9 to edit genomes in mice and human cells in 2013,\textsuperscript{359} the technology has been thought to have the greatest potential to cure some of the deadly diseases that humans cannot control today, including against bacteria-plagued diseases (i.e., CF), cancer and more importantly hereditary disorders. Although there is no therapy in clinics yet, the advance in off-target control and improved editing efficiency will translate gene therapy from bench to bedside in the foreseeable future. Both the gene editing and nucleic acid-based antibiotics need to be delivered into the host and cross spontaneously bacterial cell walls and membranes.

The major strategies for gene delivery are viral and non-viral vector systems. Non-viral strategy includes electroporation (direct injection, in vitro), lipids, and polymers. Research has also shown that LPN (Liposome polymer nucleic acid) is a versatile platform for efficiently delivering diverse nucleic acids to Gram-negative bacteria.\textsuperscript{358} Intracellular delivery of LPN can be directed against essential genes, resulting in bacterial growth inhibition or death.\textsuperscript{360} For viral delivery strategy, lentivirus, adenovirus-associated virus (AAV), and adenovirus vectors are often used.\textsuperscript{61} The lentiviral vector can efficiently integrate a foreign gene into the host chromosome, thereby achieving the effect of persistently expressing the sequence of interest. For some cells that are difficult to transfect, such as primary cells, stem cells, undifferentiated cells, etc., the use of lentiviral vectors has shown versatility to greatly improve the transduction efficiency of the target gene or the target shRNA. Therefore, in vitro and in vivo experiments, lentivirus has become one of the commonly used forms of expression for expressing foreign genes or exogenous shRNAs, and is gaining increasing attention.\textsuperscript{362} AAV's function mechanism is very similar to lentiviruses. Compared to the lentiviruses, AAV shows better safety, a wide range of host cells, and low immunogenicity, so it has become the most promising gene transfer vector, especially for the CRISPR system.\textsuperscript{363,364} The AAV-based gene delivery may bear high transduction efficiency for delivering nucleic acid-based antibiotics, leading to better therapeutic effects.

Development of vaccines is painfully slow

Nip in the bud, vaccines have been a viable approach for preventing millions of people from suffering devastating pandemic infections. Vaccine formulations can be efficiently targeted by antigen-presenting cells to generate both humoral and cellular immune responses.

Only can a few of vaccines that have being tested pass the hurdles of clinical trials to inoculate human populations. A major component of the bacterium targeted by vaccines are LPS and OMP with high rates of variations and mutations, which present challenges in vaccine designs. Vaccines based on these components have shown effects against homologous strains, but no effect against different serotypes. Two strategies have been adopted with LPS-vaccines: the first was to use lipid droplets for carrying LPS components into the host; and the second was stripping the lipid portion off LPS to create serotype-specific protection, which is considered the best option.\textsuperscript{365} Proteins or genetic vaccines appear to be the best candidates to vaccinate for \textit{P. aeruginosa}. Emerging strains of \textit{P. aeruginosa} are a key model system for investigating T4P structure and function, which may provide insight into vaccine design. Research indicates a serious nosocomial pathogen with facile growth requirements or contribution to motility.\textsuperscript{366} Flagellin and flagella have high immunogenicity, therefore, they are potential targets for vaccination against \textit{P. aeruginosa}.\textsuperscript{367} The use of flagella is not only for motility purpose, but also for assisting attachment to the respiratory epithelium and activating TLR5. \textit{P. aeruginosa} flagellin is a major unit of flagellar filament that is required to target protective antibodies and virulence. Flagella vaccinated seem to help target acute infections and provide substantial relief of disease by preventing bacterial invasions and spreads. \textit{Klebsiella} surface O polysaccharides (OPS) are protective antigens against infection in animals. The development of combined \textit{K. pneumoniae} and \textit{P. aeruginosa} glycoconjugate vaccine of four common \textit{Klebsiella} OPS types may improve the control of human infections. OPS is chemically associated with two flagellin types, FlaA and FlaB.\textsuperscript{368} Monoclonal antibodies as passive immunity have shown protection of susceptible individuals to delay or prevent initial infections, especially those with cystic fibrosis.\textsuperscript{369} Increasing vaccines targeting iron chelation, lectin inhibitors, QS inhibition, have been developed. Finally, OMVs are being tested in clinical settings either vaccines or therapeutic carriers.\textsuperscript{58} Taken together, despite the strong interest in research and urgent need in public health, vaccines against \textit{P. aeruginosa} are still unavailable for clinical application.

**FUTURE DIRECTIONS**

In the past several decades, although human beings have witnessed great scientific advances in basic research of pathogenesis and host defense as well as preventative and therapeutic development with \textit{P. aeruginosa}, our understanding of this bacterium is still limited, impeding the hunt for novel effective
therapeutic in the clinical settings. There are a large number of questions requiring clarification. (1) How do drug-resistant invasive strains emerge in the context with abuse of antibiotics or normal evolution? (2) How many other virulence factors are not discovered and characterized but critical for bacterial pathogenesis and evolution, especially those with remarkable virulence? (3) How the elements of the bacterium trigger and how the bacterium evade the activation, phagocytosis, and clearance by the human immune system? (4) What are new mechanisms responsible for increasing resistance in antimicrobial therapy? (5) How to better identify new host factors for improved immunity against this bacterium? (6) Can the scientific community establish a systematic and all-inclusive guideline to facilitate the discovery and revelation of novel antimicrobials, immune modulators, and other disease-modifying therapeutics?

**SUMMARY**

As an opportunistic pathogen, *P. aeruginosa* has a complex regulatory system that is closely connected and mutually regulated to cope with the harsh external and internal environment, which causes substantial morbidity, debilitating diseases, shortened life span, and high mortality in humans (Fig. 7). In this comprehensive review and other articles, scientists have discussed the virulence factors of *P. aeruginosa*, such as LPS, adherence factors, elastase, secretion systems, and OMVs. We also introduced the recent progress in new antibiotic formulations and compounds, phage therapy strategy, vaccine approaches, nanoparticle fabrication as well as gene editing and nucleic acid-based antibiotics. Furthermore, we have included a large set of immune responses from hosts, including cell types, innate and adaptive immunity, and emerging advances in immunological research.
Our paper is a rarely extensive review that covers both bacterial pathogenesis and host defense in a great depth, serving as an irreplaceable reference for both students, doctors, and scientists who want to better understand P. aeruginosa. This comprehensive and analytic summary of current literature may enrich our knowledge in the balance between P. aeruginosa invasion and host responses. Despite the vast progress made over the years, a number of questions ranging from basic to clinical and applied aspects remain to be answered and further increased research efforts are still needed to study P. aeruginosa, which will improve our design to more effectively combat the infection caused by emerging drug-resistance strains.

ACKNOWLEDGEMENTS
This work was supported by National Institutes of Health Grants R01 AI09317-06 and AI138203-3 to M.W. Some icons or graphic element in the Figures (Figs. 1–7) are adapted from BioRender.com (2022). Retrieved from https://app.biorender.com/. Final schematic illustrations were created and integrated by our original design.

AUTHOR CONTRIBUTIONS
M.W. conceived, supervised, and revised the paper; S.Q. organized and analytic summary of current literature may enrich our design to more effectively combat the infection caused by emerging drug-resistance strains.

ADDITIONAL INFORMATION
Competing interests: The authors have no financial conflict of interest. Xiangrong Song and Min Wu are editorial board members of Signal Transduction and Targeted Therapy, but they have not been involved in the process of the manuscript handling.

REFERENCES
1. Rossi, E. et al. Pseudomonas aeruginosa adaptation and evolution in patients with cystic fibrosis. Nat. Rev. Microbiol. 19, 331–342 (2021).
2. Jurado-Martín, I., Sainz-Mejias, M. & McClean, S. Pseudomonas aeruginosa: an audacious pathogen with an adaptable arsenal of virulence factors. Int. J. Mol. Sci. 22, 1–35 (2021).
3. Cendra, M. D. M. & Torrents, E. Pseudomonas aeruginosa biofilms and their partners in crime. Biotechnol. Adv. 49, 107734 (2021).
4. Sinha, M. et al. Pseudomonas aeruginosa theft biofilm require host liquids of cutaneous wound. Ann. Surg. 5252, 1–23 (2021).
5. Tang, P. et al. BNT162b2 and mRNA-1273 COVID-19 vaccine effectiveness against the SARS-CoV-2 Delta variant in Qatar. Nat. Med. 27, 2136–2143 (2021).
6. Blomquist, K. C. & Nix, D. E. A critical evaluation of newer beta-lactam antibiotics for treatment of Pseudomonas aeruginosa infections. Ann. Pharmacother. 55, 1010–1024 (2021).
7. Jangra, V., Sharma, N. & Chhillar, A. K. Therapeutic approaches for combating Pseudomonas aeruginosa Infections. Microbes Infect. 24, 104950 (2022).
8. Daikos, G. L. et al. Review of ceftazidime-avibactam for the treatment of infections caused by Pseudomonas aeruginosa. Antibiotics. 10, 1–24 (2021).
9. Botelho, J., Grossa, F. & Peixe, L. Antibiotic resistance in Pseudomonas aeruginosa—mechanisms, epidemiology and evolution. Drug Resist. Update. 44, 10064 (2019).
10. Haque, M., Sartelli, M., McKimm, J. & Abu Bakar, M. Health care-associated infections—an overview. Infect. Drug Resist. 11, 2321–2333 (2018).
11. Lopez-Calleja, A. et al. Antimicrobial activity of cetofloxazone-tazobactam against multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa clinical isolates from a Spanish hospital. Rev. Esp. Quimioter. 32, 68–72 (2019).
12. European Centre for Disease Prevention and Control publishes Annual epidemiological report 2011. Euro. Surveill. 16, 20012 (2011).
13. McCarthy, K. L. & Paterson, D. L. Increased risk of death with recurrent Pseudomonas aeruginosa bacteremia. Diagn. Microbiol. Infect. Dis. 88, 152–157 (2017).
14. Feng, W. et al. Epidemiology and resistance characteristics of Pseudomonas aeruginosa isolates from the respiratory department of a hospital in China. J. Glob. Antimicrob. Resist. 8, 142–147 (2017).
15. Xin, X. F., Kvitko, B. & He, S. Y. Pseudomonas syringae: what it takes to be a pathogen. Nat. Rev. Microbiol. 16, 316–328 (2018).
16. Vidaliakk, C. & Choiutimal, S. H. Pseudomonas aeruginosa in bronchiectasis: infection, inflammation, and therapies. Expert Rev. Respir. Med. 15, 649–662 (2021).
17. Park, W. S. et al. Benzyl isoioxyanate attenuates inflammasome activation in Pseudomonas aeruginosa LPS-stimulated THP-1 cells and exerts regulation through the MAPKs/NF-kappaB pathway. Int. J. Mol. Sci. 23, 1–10 (2022).
18. Chambers, J. R., Cherry, K. E. & Sauer, K. Susceptibility of Pseudomonas aeruginosa dispersed cells to antimicrobial agents is dependent on the dispersion cue and class of the antimicrobial agent used. Antimicrob. Agents Chemother. 61, 1–18 (2017).
19. Sabnis, A. et al. Colistin kills bacteria by targeting lipopolysaccharide in the cytoplasmic membrane. Elife. 10, 1–26 (2021).
20. Ozer, E. et al. An inside look at a biofilm: Pseudomonas aeruginosa flagella biotracking. Sci. Adv. 7, 1–15 (2021).
21. Yang, J. J., Tsuet, K. C. & Shen, E. P. The role of Type III secretion system in the pathogenesis of Pseudomonas aeruginosa microbial keratitis. Tzu. Chi. Med. J. 54, 8–14 (2022).
22. Karash, S., Nordell, R., Ozer, E. A. & Yahr, T. L. Genome sequences of two Pseudomonas aeruginosa isolates with defects in type III secretion system gene expression from a chronic ankle wound infection. Microbiol. Spectr. 9, e0034021 (2021).
23. Alaraktchi, F. A., Svendsen, W. E. & Molin, S. Electrochemical detection of pyocyanin as a biomarker for Pseudomonas aeruginosa: a focused review. Sensors. 20, 1–15 (2020).
24. Chadha, J., Harjai, K. & Chhibber, S. Revisiting the virulence hallmarks of Pseudomonas aeruginosa: a chronicle through the perspective of quorum sensing. Environ. Microbiol. 00, 1–27 (2021).
25. Zhao, F., Wang, Q., Zhang, Y. & Lei, L. Anaerobic biosynthesis of rhamnolipids by Pseudomonas aeruginosa: performance, mechanism and its application potential for enhanced oil recovery. Micro. Cell Fact. 20, 103 (2021).
26. Perras, Q. et al. Opportunistic use of catecholamines neurotransmitters as siderophores to access iron by Pseudomonas aeruginosa. Environ. Microbiol. 24, 878–893 (2022).
27. Dar, H. H. et al. A new thiol-independent mechanism of epithelial host defense against Pseudomonas aeruginosa: INOS/NO\(^{\bullet}\) sabotrage of death-ferrroptosis. Redox. Biol. 45, 102045 (2021).
28. Maldonado, R. F., Sa-Correia, I. & Valvano, M. A. Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiol. Rev. 40, 480–493 (2016).
29. Cross, A. S. Anti-endotoxin vaccines: back to the future. Virulence. 5, 219–225 (2014).
30. Goldberg, J. B. & Pler, G. B. Pseudomonas aeruginosa lipopolysaccharides and pathogenesis. Trends Microbiol. 4, 490–494 (1996).
31. Amarante-Mendes, G. P. et al. Pattern recognition receptors and the host cell death molecular machinery. Front. Immunol. 9, 2379 (2018).
32. Mogensen, T. H. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin. Microbiol. Rev. 22, 240–273 (2009).
33. Li, D. & Wu, M. Pattern recognition receptors in health and diseases. Signal Transduc. Target Ther. 6, 291 (2021).
34. Nadanats, Y. et al. NOD-like receptor protein 3 inflammasome priming and activation in Barrett’s epithelial cells. Cell Mol. Gastroenterol. Hepatol. 2, 439–453 (2016).
35. Sebastian-Valverde, M. & Pasinetti, G. M. The NLRP3 inflammasome as a critical actor in the inflamming process. Cells. 9, 1–28 (2020).
36. Stephens, M. & von der Weid, P. Y. Lipopolysaccharides mediate intestinal epithelial permeability and inflammation in a species-specific manner. Gut Microbes. 11, 421–432 (2020).
37. Liu, W., Sun, T. & Wang, Y. Integrin alphabeta6 mediates epithelial-mesenchymal transition in human bronchial epithelial cells induced by lipopolysaccharides of Pseudomonas aeruginosa via TGF-beta1-Smad2/3 signaling pathway. Folia Microbiol. 65, 329–338 (2020).
38. Ramjeet, M. et al. Truncation of the lipopolysaccharide outer core affects susceptibility to antimicrobial peptides and virulence of Actinobacillus pleur- opneumoniae serotype 1. J. Biol. Chem. 280, 39104–39114 (2005).
39. Murray, G. L. et al. Mutations affecting Leptospira interrogans lipopolysaccharide, which will improve our design to more effectively combat the infection caused by emerging drug-resistance strains.

Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic... Qin et al.
20

45. Kim, J. Y. et al. Engineered bacterial outer membrane vesicles with enhanced functionality. J. Mol. Biol. 380, 51–66 (2008).

46. Jan, A. T. Outer membrane vesicles (OMVs) of Gram-negative bacteria: a prospective update. Front. Microbiol. 8, 1053 (2017).

47. Bomberger, J. M. et al. Long-distance delivery of bacterial virulence factors by Pseudomonas aeruginosa outer membrane vesicles. PLoS Pathog. 5, e1000382 (2009).

48. Kadurugamuwa, J. L. & Beveridge, T. J. Virulence factors are released from Pseudomonas aeruginosa in association with membrane vesicles during normal growth and exposure to gentamicin: a novel mechanism of enzyme secretion. J. Bacteriol. 177, 3998–4008 (1995).

49. Kato, S., Kowashi, Y. & Demuth, D. R. Outer membrane-like vesicles secreted by Actinobacillus actinomycetemcomitans are enriched in leukotriene. Micro. Pathog. 32, 1–13 (2002).

50. Wai, S. N. et al. Vesicle-mediated export and assembly of pore-forming oligomers of the enterobacterial Cya cytotoxin. Cell. 115, 25–35 (2003).

51. Kesty, N. C. & Kuehn, M. J. Incorporation of heterologous outer membrane and periplasmic proteins into Escherichia coli outer membrane vesicles. J. Biol. Chem. 279, 2069–2076 (2004).

52. Bomberger, J. M. et al. A Pseudomonas aeruginosa toxin that hijacks the host ubiquitin proteolytic system. PLoS Pathog. 7, e1001325 (2011).

53. Bauman, S. J. & Kuehn, M. J. Purification of outer membrane vesicles from Pseudomonas aeruginosa and their activation of an IL-8 response. Microbes Infect. 8, 2400–2408 (2006).

54. Kuepfer, K. et al. A novel mechanism of host-pathogen interaction through secretion of RNA in bacterial outer membrane vesicles. PLoS Pathog. 12, e1005672 (2016).

55. Ballok, A. E. et al. Epoxide-mediated differential packaging of Cif and other virulence factors into outer membrane vesicles. J. Bacteriol. 196, 3633–3642 (2014).

56. Stanton, B. A. Effects of Pseudomonas aeruginosa on CFTR chloride secretion and the host immune response. Am. J. Physiol. Cell Physiol. 312, C357–C366 (2017).

57. Furuya, N. & Sircili, M. P. Outer membrane vesicles (OMVs) produced by Gram-negative bacteria: structure, functions, biogenesis, and vaccine application. Biomed. Res. Int. 2021, 1490732 (2021).

58. van der Pol, L., Stork, M. & van der Ley, P. Outer membrane vesicles as platform vaccine technology. Biotechnol. J. 10, 1689–1706 (2015).

59. Balhuizen, M. D., Veldhuizen, E. J. A. & Haagsman, H. P. Outer membrane vesicle formation but not environmental adaption in Pseudomonas aeruginosa strain PAO1 is regulated by quorum sensing and Fur and modulates type VI secretion locus I and homologous loci II and III, which are required for virulence in Pseudomonas aeruginosa. Mol. Microbiol. 54, 1090–1103 (2004).

60. Ventre, I. et al. Multiple sensors control reciprocal expression of Pseudomonas aeruginosa regulatory RNA and virulence genes. Proc. Natl Acad. Sci. USA. 103, 171–176 (2006).

61. Goodman, A. L. et al. Direct interaction between sensor kinase proteins mediates acute and chronic disease phenotypes in a bacterial pathogen. Genes Dev. 23, 249–259 (2009).

62. Chakravarty, S. et al. Pseudomonas aeruginosa magnesium transporter MgtE inhibits type III secretion system gene expression by stimulating rsmY transcription. J. Bacteriol. 199, e00268–17 (2017).

63. Chen, L., Zou, Y., Kronf, A. A. & Wu, Y. Type VI secretion system of Pseudomonas aeruginosa is associated with biofilm formation but not environmental adaptation. Microbiolog. 9, e991 (2020).

64. Rasko, D. A. & Sperandio, V. Anti-virulence strategies to combat bacteria-mediated disease. Nat. Rev. Drug Discov. 9, 117–128 (2010).

65. Sharma, A. K. et al. Bacterial virulence factors: secrets for survival. Indian J. Microbiol. 57, 1–10 (2017).

66. Filloux, A. Protein secretion systems in Pseudomonas aeruginosa: an essay on diversity, evolution, and function. Front. Microbiol. 2, 155 (2011).

67. de Sousa, T. et al. Genomic and metabolic characteristics of the pathogenicity in Pseudomonas aeruginosa. Int. J. Mol. Sci. 22, 1–28 (2021).

68. Thomas, S., Holland, I. B. & Schmitt, L. The Type 1 secretion pathway—the hemolysin system and beyond. Biochim. Biophys. Acta. 1843, 1629–1641 (2014).

69. Smith, T. J., Sondermann, H. & O'Toole, G. A. Type I does the two-step: type I secretion substrates with a functional periplasmic intermediate. J. Bacteriol. 200, e01668–18 (2018).

70. Alav, I. et al. Structure, assembly, and function of tripartite efflux and type I secretion systems in Gram-negative bacteria. Chem. Rev. 121, 5479–5596 (2021).

71. Cassat, J. E. & Skare, E. P. Iron in infection and immunity. Cell Host Microbe. 13, 509–519 (2013).

72. Yahr, T. L., Goranson, J. & Frank, D. W. Exoenzyme S of Pseudomonas aeruginosa in infection by a type III pathway. Mol. Microbiol. 22, 991–1003 (1996).

73. Jimenez, P. N. et al. The multiple signaling systems regulating virulence in Pseudomonas aeruginosa. Microbiol. Mol. Biol. Rev. 76, 46–65 (2012).

74. Sosic, C. et al. Cross talk between type III secretion and flagellar assembly systems in Pseudomonas aeruginosa. J. Bacteriol. 189, 3124–3132 (2007).

75. Hauser, A. R. The type III secretion system of Pseudomonas aeruginosa: infection by injection. Nat. Rev. Microbiol. 7, 654–665 (2009).

76. Coburn, B., Sekirov, I. & Finlay, B. B. Type III secretion systems and disease. Clin. Microbiol. Rev. 20, 535–549 (2007).

77. Francis, M. S., Wofl-Watz, H. & Forsberg, A. Regulation of type III secretion systems. Curr. Opin. Microbiol. 5, 166–172 (2002).

78. Zhu, M. et al. Modulation of type III secretion system in Pseudomonas aeruginosa: involvement of the PA4857 gene product. Front. Microbiol. 7, 17 (2016).
104. Han, Y. et al. A Pseudomonas aeruginosa type VI secretion system regulated by CueR facilitates copper acquisition. PLoS Pathog. 15, e1008198 (2019).

105. Gao, P. et al. OprC impairs host defense by increasing the quorum-sensing-mediated virulence of Pseudomonas aeruginosa. Front. Immunol. 11, 1696 (2020).

106. Wang, T. et al. A type VI secretion system delivers a cell wall amide to target bacterial competitors. Mol. Microbiol. 114, 308–321 (2020).

107. Shao, X. et al. Novel therapeutic strategies for treating Pseudomonas aeruginosa infection. Expert Opin. Drug Discov. 15, 1403–1423 (2020).

108. Horna, G. & Ruiz, J. Type 3 secretion system of Pseudomonas aeruginosa. Microbiol. Res. 246, 126719 (2021).

109. Sana, T. G., Berni, B. & Bleves, S. The T6SSs of Pseudomonas aeruginosa strain PA01 and their effectors: beyond bacterial-cell targeting. Front. Cell Infect. Microbiol. 6, 61 (2020).

110. Records, A. R. & Gross, D. C. Sensor kinases ResT and LaSd regulate Pseudomonas syringae type VI secretion and virulence factors. J. Bacteriol. 192, 3584–3596 (2010).

111. Dadashi, M. et al. Putative RNA ligase RtcB affects the Switch between T6SS and T3SS in Pseudomonas aeruginosa. Int. J. Mol. Sci. 22, 1–21 (2021).

112. Xia, Y. et al. YbeY controls the atypical anoikis apoptosis in target host cells by transforming Crk adaptor protein into a cytotoxin. FEBS J. 282, 685–695 (2021).

113. Diaz, M. R., King, J. M. & Yahr, T. L. Intrinsic and extrinsic regulation of type III secretion systems. Front. Cell Infect. Microbiol. 2, 89 (2011).

114. Kambelis, J. et al. Pseudomonas aeruginosa ExoS induces intrinsic apoptosis in target host cells in a manner that is dependent on its GAP domain activity. Sci. Rep. 8, 14047 (2018).

115. Kroken, A. R. et al. Exotoxin S secreted by internalized Pseudomonas aeruginosa delays lytic host cell death. PLoS Pathog. 18, e1010306 (2022).

116. Jia, J., Wang, Y., Zhou, L. & Jin, S. Expression of Pseudomonas aeruginosa toxin ExoC effectively induces apoptosis in host cells. Infect. Immun. 74, 6557–6570 (2006).

117. Finck-Barbancon, V. & Frank, D. W. Multiple domains are required for the toxic activity of Pseudomonas aeruginosa ExoA. J. Bacteriol. 183, 4330–4344 (2001).

118. Hardy, K. S. et al. ExoU induces lung endothelial cell damage and activates proinflammatory caspase-1 during Pseudomonas aeruginosa infection. Toxins. 14, 152 (2022).

119. Sutterwala, F. S. et al. Immune recognition of Pseudomonas aeruginosa mediated by the IFAP/NLRCA inflammasome. J. Exp. Med. 204, 3235–3245 (2007).

120. Lindsey, A. S. et al. Analysis of pulmonary vascular injury and repair during Pseudomonas aeruginosa infection-induced pneumonia and acute respiratory distress syndrome. Pulm. Circ. 9, 2045894019862941 (2019).

121. Foulkes, D. M. et al. Pseudomonas aeruginosa toxin ExoU is a therapeutic candidate in the treatment of bacterial infections. Microorganism. 7, 707 (2019).

122. Wood, S., Goldufsky, J. & Bally, M. G. Exposition-dependent virulence of Pseudomonas aeruginosa strain PA01 and their effectors: beyond bacterial-cell targeting. Front. Cell Infect. Microbiol. 6, 2020.

123. Mohamed, M. F. et al. Pseudomonas aeruginosa ExoU induces G1 cell cycle arrest in melanoma cells. Cell Microbiol. 23, e13339 (2021).

124. Wisnioski, G. L. et al. Enhanced annotations and features for comparing thousands of Pseudomonas genomes in the Pseudomonas genome database. Nucleic Acids Res. 44, D646–D653 (2016).

125. He, C. et al. Bacterial nucleotidyl cyclase inhibits the host innate immune response by suppressing TAK1 activation. Infect. Immun. 85, e00239–17 (2017).

126. Jeon, J., Kim, Y. J., Shin, H. & Ha, U. H. T3SS effector ExoY reduces inflammasome-related responses by suppressing bacterial motility and delaying activation of NF-kappaB and caspase-1. FEBS J. 284, 3392–3403 (2017).

127. Yang, X., Long, M. & Shen, X. Effector-Immunity pairs provide the T3SS nano-machine its offensive and defensive capabilities. Molecules. 23, 1009 (2018).

128. Russell, A. B. et al. Type VI secretion delivers bacteriocidal effectors to target cells. Nature. 475, 343–347 (2011).

129. Pérez-Lorente, A. J., Molina-Santiago, C., de Vicente, A. & Romero, D. Sporulation-mediated virulence of Pseudomonas aeruginosa strain PA01. Mol. Microbiol. 26, 60, e00127 (2015).

130. Rollman, J. M., Merdes, A., Mooney, L. & Agard, D. A. Microtubule nucleation by gamma-tubulin complexes. Nat. Rev. Mol. Cell Biol. 12, 709–721 (2011).

131. Kierbel, A., Gassama-Diagne, A., Mostov, K. & Engel, J. N. The phosphoinositide-3-kinase-protein kinase B/Akt pathway is critical for Pseudomonas aeruginosa strain PAK internalization. Mol. Biol. Cell. 16, 2577–2585 (2005).

132. Naskar, S., Hohl, M., Tassanari, M. & Low, H. H. The structure and mechanism of the bacterial type II secretion system. Mol. Microbiol. 115, 412–424 (2021).

133. Liu, P. A., Fullman-Elcog, D. C. & Georgiou, G. The bacterial twin-arginine translocation pathway. Annu. Rev. Microbiol. 60, 373–395 (2006).

134. Qin et al. Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic...
22

Sadikot, R. T., Blackwell, T. S., Christman, J. W. & Prince, A. S. Pathogen-host interactions in Pseudomonas aeruginosa pneumonia. Am. J. Respir. Crit. Care Med. 179, 1209–1223 (2005).

Blus-Kadoš, I., Zilka, A., Yerushalmi, G. & Banin, E. The effect of psst and phoB on quorum sensing and swarming motility in Pseudomonas aeruginosa. PLoS One. 8, e74444 (2013).

Coolie, M., Chhabra, S. R. & Williams, P. N-Acylhomoserine lactone-mediated quorum sensing: a twist in the tail and a blow for host immunity. Chem. Biol. 15, 1141–1147 (2008).

Schuster, M., Sexton, D. J., Diggle, S. P. & Greenberg, E. P. Acyl-homoserine lactone quorum sensing: from evolution to application. Annu. Rev. Microbiol. 67, 63–83 (2013).

Venturi, V. Regulation of quorum sensing in Pseudomonas. FEMS Microbiol. Rev. 30, 274–291 (2006).

Welsh, M. A. & Blackwell, H. E. Chemical probes of quorum sensing: from compound development to biological discovery. FEMS Microbiol. Rev. 40, 774–794 (2016).

Lee, J. & Zhang, L. The hierarchy quorum sensing network in Pseudomonas aeruginosa. Protein Cell. 6, 26–41 (2015).

Li, S., Felder, M. J. Exploiting quorum sensing to confuse bacterial pathogens. Microbiol. Mol. Biol. Rev. 77, 113–111 (2013).

Schuster, M. & Greenberg, E. P. Early activation of quorum sensing in Pseudomonas aeruginosa reveals the architecture of a complex regulon. BMC Genomics. 8, 287 (2007).

Fuqua, C. The QscR quorum-sensing regulator of Pseudomonas aeruginosa: an orphan receptor claims its identity. J. Bacteriol. 188, 3169–3171 (2006).

Li, Y., H., et al. Molecular mechanisms of master regulator VqsP mediating quorum-sensing and antibiotic resistance in Pseudomonas aeruginosa. Nucleic Acids Res. 42, 10307–10320 (2014).

Diggle, S. P. et al. Advancing the quorum in Pseudomonas aeruginosa: MvfA and the regulation of N-acylhomoserine lactone production and virulence gene expression. J. Bacteriol. 184, 2576–2586 (2002).

Castang, S., McManus, H. R., Turner, K. H. & Dove, S. L. H-NS family members function coordinately in an opportunistic pathogen. Proc. Natl Acad. Sci. USA. 105, 18947–18952 (2008).

Rampioni, G. et al. Contribution of the RsaL global regulator to Pseudomonas aeruginosa virulence and biofilm formation. FEMS Microbiol. Lett. 301, 210–217 (2009).

Rampioni, G. et al. RsaL provides quorum sensing homeostasis and functions as a global regulator of gene expression in Pseudomonas aeruginosa. Mol. Microbiol. 66, 1557–1565 (2007).

Kang, H. et al. Crystal structure of Pseudomonas aeruginosa RsaL bound to promoter DNA reaffirms its role as a global regulator involved in quorum-sensing. Nucleic Acids Res. 45, 699–710 (2017).

Balasubramanian, D., et al. The regulatory repertoire of Pseudomonas aeruginosa AmpC ss-lactamase regulator AmpR includes virulence genes. PLoS One. 7, e34067 (2012).

Zhao, J. et al. Structural and molecular mechanism of CdpR involved in quorum-sensing and bacterial virulence in Pseudomonas aeruginosa. PLoS Biol. 14, e1002499 (2016).

Cao, Q. et al. A novel signal transduction pathway that modulates rhl quorum sensing and bacterial virulence in Pseudomonas aeruginosa. PLoS Pathog. 10, e1004304 (2014).

Yang, N. et al. The Crc protein participates in down-regulation of the Lon gene in Pseudomonas aeruginosa. FEMS Microbiol. Lett. 1659–1671 (2013).

Mikkelsen, H., McMullan, R. & Filloux, A. The Pseudomonas aeruginosa reference strain PA14 displays increased virulence due to a mutation in ldsL. PLoS One. 6, e29113 (2011).

Kazmierzak, B. I., Schniederbernd, M. & Jain, R. Cross-regulation of Pseudomonas motility systems: the intimate relationship between flagella, pili and virulence. Curr. Opin. Microbiol. 28, 78–82 (2015).

Rao, F., Yang, Y., Qi, Y. & Liang, Z. X. Catalytic mechanism of cyclic di-GMP synthetase Upp2: a novel copper-sensing two-component system for inducing Dsb KinB in Pseudomonas aeruginosa. J. Biol. Chem. 287, 1688 (2006).

Francis, V. I., Stevenson, E. C. & Porter, S. L. Two-component systems required for pathogenesis, virulence factors, antibiotic resistance and biofilm formation in Pseudomonas aeruginosa. FEMS Microbiol. Lett. 375, 3759 (2018).

Mikkelsen, H., Hui, K., Barraud, N. & Filloux, A. The pathogenicity island encoded PvrSR/RcsCB regulatory network controls biofilm formation and dispersal in Pseudomonas aeruginosa PA14. Mol. Microbiol. 89, 450–463 (2013).

Gellatly, S. L. et al. The Pseudomonas aeruginosa PhoP-PhoQ two-component regulatory system is induced upon interaction with epithelial cells and controls cytotoxicity and inflammation. Infect. Immun. 80, 3122–3131 (2012).

Mulcahy, H., Charron-Mazend, L. & Lewenza, S. Extracellular DNA chelates cations and induces antibiotic resistance in Pseudomonas aeruginosa biofilms. PLoS Pathog. 4, e1000213 (2008).

Lewenza, S. Extracellular DNA-induced antimicrobial peptide resistance mechanisms in Pseudomonas aeruginosa. Front. Microbiol. 4, 21 (2013).

Turner, K. H. et al. Requirements for Pseudomonas aeruginosa acute burn and chronic surgical wound infection. PLoS Genet. 10, e1004518 (2014).

Hickman, J. W. & Hanwood, C. S. Identification of FieQ from Pseudomonas aeruginosa as a c-di-GMP-responsive transcription factor. Mol. Microbiol. 69, 376–389 (2008).

O'Toole, G. A. & Wong, G. C. Sensational biofilms: surface sensing in bacteria. eLife. 3, e03998 (2014).

Luo, Y. et al. A hierarchical cascade of second messengers regulates Pseudomonas aeruginosa surface behaviors. MBio. 6, e02456–14 (2015).

Cao, Q. et al. Mutation-induced remodeling of the BfmRS regulatory network system is induced upon interaction with epithelial cells and controls cytotoxicity and inflammation. Infect. Immun. 89, 107–1019 (2014).

Pita, T., Feliciano, J. R. & Leitao, J. H. Small noncoding regulatory RNAs from Pseudomonas aeruginosa and Burkholderia cepacia complex. Int. J. Mol. Sci. 19, 3759 (2018).

Sonkleter, E. et al. Reduced virulence of a hfl mutant of Pseudomonas aeruginosa O1. Micro. Pathog. 35, 217–228 (2003).

Zhao, K., Li, Y., Yue, B. & Wu, M. Genes as early responders regulate quorum-sensing and bacterial cooperation in Pseudomonas aeruginosa. PLoS One. 9, e101887 (2014).

Gomez-Lozano, M. et al. Diversity of small RNAs expressed in Pseudomonas species. Environ. Microbiol. Rep. 7, 227–236 (2015).
Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic... Qin et al.
Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic... Qin et al.

282. Wu, M. et al. Host DNA repair proteins in response to Pseudomonas aeruginosa in lung epithelial cells and in mice. Infect. Immun. 79, 75–87 (2011).

283. Huang, T. et al. MicroRNA-302-367 cluster impacts host antimicrobial defense via regulation of mitophagic response against Pseudomonas aeruginosa infection. Front. Immunol. 11, 569173 (2020).

284. Hayashi, F. et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature. 410, 1099–1103 (2001).

285. Barton, G. M., Kagan, J. C. & Medzhitov, R. Intracellular localization of Toll-like receptor 9 prevents recognition of self DNA but facilitates access to viral DNA. Nat. Immunol. 7, 49–56 (2006).

286. Martinon, F., Burns, K. & Tschopp, J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of pro-β-caspase-1. Nature. 420, 488–492 (2002).

287. Sharma, D. & Kanneganti, T. D. The cell biology of inflammasomes: mechanisms of inflammasome activation and regulation. J. Cell Biol. 213, 617–629 (2016).

288. Shi, J. et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature. 526, 660–665 (2015).

289. Liu, X. et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature. 535, 153–158 (2016).

290. Heussler, G. E. et al. Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas systems: a molecular platform for acquiring and expelling mobile genetic elements. Annu. Rev. Genet. 43, 1048–10860 (2015).

291. Pang, Z., Sun, G., Junkins, R. D. & Lin, T. J. AIM2 in Pseudomonas aeruginosa infection: protectivity in murine model. Nat. Commun. 4, 493–500 (2013).

292. Kuo, E. A. et al. Inflammasome detection of the type III secretion apparatus. Nat. Microbiol. 4, 242–248 (2019).

293. Zhao, Y. et al. The NLRC4 inflammasome in Pseudomonas aeruginosa infection: naturally occurring and engineered phages. J. Bacteriol. 194, 5728–5732 (2012).

294. Louwen, R. et al. The role of CRISPR-Cas systems in virulence of pathogenic bacteria. Microbiol. Mol. Biol. Rev. 78, 74–88 (2014).

295. Li, R. et al. Type I CRISPR-Cas targets endogenous genes and regulates virulence to evade mammalian host immunity. Cell Res. 26, 1273–1287 (2016).

296. Hoyland-Krogshbo, N. M. et al. Quorum sensing controls the Pseudomonas aeruginosa CRISPR-Cas adaptive immune system. Proc. Natl Acad. Sci. USA. 114, 131–135 (2017).

297. Alesht, E. O. et al. Bacterial biodiversity drives the evolution of CRISPR-based phage resistance. Nature. 574, 549–552 (2019).

298. Vasquez-Rifo, A. et al. The Pseudomonas aeruginosa accessory genome elements influence virulence towards Caenorhabditis elegans. Genome Biol. 20, 270 (2019).

299. Neumueller, G. E. et al. Clustered regularly interspaced short palindromic repeat-dependent, biofilm-specific death of Pseudomonas aeruginosa mediated by increased expression of phage-related genes. mBio. 6, e00129–00115 (2015).

300. Sampson, T. R. et al. A CRISPR-Cas system enhances envelope integrity mediating antibiotic resistance and inflammasome evasion. Proc. Natl Acad. Sci. USA. 111, 11665–11668 (2014).

301. Pawluk, A. et al. Inactivation of CRISPR-Cas systems by anti-CRISPR proteins in diverse bacterial species. Nat. Microbiol. 1, 16085 (2016).

302. Bondy-Denomy, J., Pawluk, A., Maxwell, K. L. & Davidson, A. R. Bacteriophage genes that inactivate the CRISPR-Cas bacterial immune system. Nature. 493, 432–439 (2013).

303. Pawluk, A. et al. Naturally occurring off-switches for CRISPR-Cas9. Cell. 167, 1829–1838, e1829 (2016).

304. Harrington, L. B. et al. A broad-spectrum inhibitor of CRISPR-Cas9. Cell. 170, 1224–1233, e1215 (2017).

305. Rauch, B. J. et al. Inhibition of CRISPR-Cas9 with bacteriophage proteins. Cell. 168, 150–158, e1010 (2017).

306. Waters, K. E. et al. Systematic discovery of natural CRISPR-Cas12a inhibitors. Science. 362, 236–239 (2018).

307. Lin, P. et al. CRISPR-Cas13 inhibitors block RNA editing in bacteria and mammalian cells. Mol. Cell. 78, 850–861, e855 (2020).

308. Lin, P. et al. Type III CRISPR-based RNA editing for programmable control of SARS-CoV-2 and human coronaviruses. Nucleic. Acids Res. 50, e47 (2022).

309. Chin, W. et al. A macromolecular approach to eradicate multidrug resistant bacterial infections while mitigating drug resistance onset. Nat. Commun. 9, 917 (2018).

310. Biswaro, L. S. et al. Antimicrobial peptides and nanotechnology, recent advances and challenges. Front. Microbiol. 9, 855 (2018).

311. Eljaaly, K. et al. Plazomicin: a novel aminoglycoside for the treatment of resistant bacteria and enhance biofilm disruption. Antimicrob. Agents Chemother. 55, 3047–3052 (2011).

312. Del Tordello, E., Danilchanka, O., McCluskey, A. J. & Mekalanos, J. J. Type VI secretion system: ROS as trigger or effector? Annu. Rev. Biochem. 87, 105–122 (2018).

313. Brien, W., Fyfe, C. & Grossman, T. H. Antibacterial activity of flagellin is mediated by Toll-like receptor 5. Front. Microbiol. 5, 122–129 (2014).

314. Zhang, F., Wen, Y. & Guo, X. CRISPR-Cas9 for genome editing: progress, implications and challenges. Hum. Mol. Genet. 23, R40–R46 (2014).

315. Makarova, K. S. et al. An updated evolutionary classification of CRISPR-Cas systems. Nat. Rev. Microbiol. 13, 722–736 (2015).

316. Koonin, E. V., Makarova, K. S. & Zhang, F. Diversity, classification and evolution of CRISPR-Cas systems. Curr. Opin. Microbiol. 37, 67–78 (2017).

317. Stanley, S. R. & Maxwell, K. L. Phage-encoded anti-CRISPR defenses. Annu. Rev. Genet. 52, 445–464 (2018).
400. Grande, K. K., Gustin, J. K., Kessler, E. & Ohman, D. E. Identification of critical residues in the propeptide of LasA protease of Pseudomonas aeruginosa involved in the formation of a stable mature protease. J. Bacteriol. 189, 3960–3968 (2007).

401. Gupta, R. K., Dhabhar, S. & Harjai, K. A. A new biosynthetic pathway for type IV pili adhesins in Pseudomonas aeruginosa. Biofactors. 19, 129–136 (2015).

402. Gutierrez-Gomez, U., Soto-Aceves, M. P., Servin-Gonzalez, L. & Saez-Trumper, L. G. Overproduction of rhamnolipids in Pseudomonas aeruginosa PA14 by redirection of the carbon flux from polyhydroxyalkanoate synthesis and overexpression of the rhlR operon. Biotechnol. Lett. 40, 1561–1566 (2018).

403. Howie, T. R. & Iglewski, B. H. Isolation and characterization of alkaline protease-dissident mutants of Pseudomonas aeruginosa in vitro and in a mouse eye model. Infect. Immun. 43, 1058–1063 (1984).

404. Islam, H. C., Afshar, D. & Esmaili, E. Effect of Satureja khouzistanae essential oil (SKEEO) on expression of lasA and lasB genes in Pseudomonas aeruginosa. Iran. J. Microbiol. 11, 55–59 (2019).

405. Ivanov, I. E. et al. Atomic force and super-resolution microscopy support a role for LasA as a cell-surface biofilm adhesin of Pseudomonas fluorescens. Res. Microbiol. 163, 685–691 (2012).

406. Jepkorir, G. et al. Structural, NMR spectroscopic, and computational investigation of hemin loading in the hemophore HasA from Pseudomonas aeruginosa. J. Am. Chem. Soc. 132, 9857–9872 (2010).

407. Kaczmarczyk, B. I. & Engel, J. N. Pseudomonas aeruginosa ExoT acts in vivo as a GTase-activating protein for RhoA, Rac1, and Cdc42. Infect. Immun. 79, 1192–2205 (2001).

408. Kidn, T. et al. Identification of arylsulfonamides as ExoU inhibitors. Bioorg. Med. Chem. Lett. 24, 3823–3825 (2014).

409. Kim, S. et al. Pseudomonas aeruginosa bacteriophage PA1O requires type IV pili for infection and shows broad bacterial and biofilm removal activity. Appl. Environ. Microbiol. 78, 6380–6385 (2012).

410. Konig, B. et al. Role of Pseudomonas aeruginosa lipase in inflammatory mediator release from human inflammatory effector cells (platelets, granulocytes, and monocytes. Infect Immun. 64, 3252–3258 (1996).

411. Kumar, R. et al. Replacing the axial ligand tyrosine 75 or its hydrogen bond partner histidine 83 minimally affects hemin acquisition by the hemophore HasA from Pseudomonas aeruginosa. Biochemistry. 53, 2112–2125 (2014).

412. Lau, G. W., Hassett, D. J., Ran, H. & Kong, F. The role of poyacyn in Pseudomonas aeruginosa infection. Trends Mol. Med. 10, 599–606 (2004).

413. Li, W. et al. Norepinephrine represses the expression of toxA and the side-ophore genes in Pseudomonas aeruginosa. FEMS Microbiol. Lett. 299, 100–109 (2009).

414. Li, S. P. Extracellular toxins of Pseudomonas aeruginosa. J. Infect. Dis. 130, S94–S99 (1974).

415. Li, S. et al. Structural insights into the Ts65 effector protein Tse3 and the Tse3-Ts3 complex from Pseudomonas aeruginosa reveal a calcium-dependent membrane-binding mechanism. Mol. Microbiol. 92, 1092–1112 (2013).

416. Miller, L. C. et al. Development of potent inhibitors of poyacycin production in Pseudomonas aeruginosa. J. Med. Chem. 58, 1298–1306 (2015).

417. Mohammadmazami, Z. et al. Inhibitory effects of Caffeol, Carvacrol, and honey on the expression of exoS and ampC genes in multidrug-resistant Pseudomonas aeruginosa using a microplate reader assay. Biochem. Biophys. Res. Commun. 417, 129–134 (2012).

418. Mohammadzamani, Z. et al. Inhibitory effects of Cinnamaldehyde, Carvacrol, and Thymol on the expression of exoS and ampC genes in multidrug-resistant Pseudomonas aeruginosa using a microplate reader assay. Biochem. Biophys. Res. Commun. 417, 129–134 (2012).

419. Mohammadzamani, Z. et al. Inhibitory effects of Cinnamaldehyde, Carvacrol, and Thymol on the expression of exoS and ampC genes in multidrug-resistant Pseudomonas aeruginosa using a microplate reader assay. Biochem. Biophys. Res. Commun. 417, 129–134 (2012).

420. Moroz, O. V. et al. The structure of a calcium-dependent phosphoinositide-specific phospholipase C from Pseudomonas sp. 62186, the first from a Gram-negative bacterium. Acta Crystallogr D. Struct. Biol. 73, 32–44 (2017).

421. Ostroff, R. M., Wrettland, B. & Vasil, M. L. Mutations in the hemolytic-proteinase c operon result in decreased virulence of Pseudomonas aeruginosa PA01 grown under phosphate-limiting conditions. Proc. Natl Acad. Sci. USA. 102, 8006–8011 (2005).

422. Paranchych, W. et al. Fimbriae (pili): molecular basis of virulence in Pseudomonas aeruginosa. Cell. Microbiol. 13, 183–196 (2011).

423. Pier, G. B. Pseudomonas aeruginosa lipopolysaccharide: a major virulence factor, initiation of inflammation and target for effective immunity. Int. J. Med. Microbiol. 297, 277–295 (2007).

424. Qin et al. Signal Transduction and Targeted Therapy (2022) 7:199.
system and directs specific secretion of the CbpE chitin-binding protein. J. Bacteriol. 196, 2376–2386 (2014).

471. Daddaoua, A. et al. GtrS and GltR form a two-component system: the central role of 2-ketoglutarate in the expression of exotoxin A and glucose catabolic enzymes in Pseudomonas aeruginosa. Nucleic Acids Res. 42, 7654–7663 (2014).

472. Marden, J. N. et al. An unusual CsrA family member operates in series with RsmA to amplify posttranscriptional responses in Pseudomonas aeruginosa. Proc. Natl Acad. Sci. USA. 110, 15055–15060 (2013).

473. Ryan Kaler, K. M., Nix, J. C. & Schubot, F. D. RetS inhibits Pseudomonas aeruginosa biofilm formation by disrupting the canonical histidine kinase dimerization interface of GacS. J. Biol. Chem. 297, 101193 (2021).

474. Heurlier, K. et al. Positive control of swarming, rhamnolipid synthesis, and lipase production by the posttranscriptional RsmA/RsmZ system in Pseudomonas aeruginosa PA01. J. Bacteriol. 186, 2936–2945 (2004).

475. Chen, G. et al. Oligoribonuclease is required for the type III secretion system and pathogenesis of Pseudomonas aeruginosa virulence. Int. J. Mol. Sci. 22, 12152 (2021).

476. Yeung, A. T., Bains, M. & Hancock, R. E. The sensor kinase CbrA is a global regulator that modulates metabolism, virulence, and antibiotic resistance in Pseudomonas aeruginosa. J. Bacteriol. 193, 918–931 (2011).

477. Lehman, H. K. & Segal, B. H. The role of neutrophils in host defense and disease. J. Allergy Clin. Immunol. 145, 1535–1544 (2020).

478. Shin, H. S. et al. Pseudomonas aeruginosa-dependent upregulation of TLR2 influences host responses to a secondary Staphylococcus aureus infection. Pathog. Dis. 69, 149–156 (2013).