We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Antimicrobial Resistance in *Pseudomonas aeruginosa*: A Concise Review

Swaraj Mohanty, Bighneswar Baliyarsingh
and Suraja Kumar Nayak

Abstract

Pseudomonas aeruginosa is one of the common species responsible for an array of diseases in the respiratory tract, gastrointestinal tract, urinary tract, bones, joints and different systemic infections of normal and immunocompromised patients as well. It exhibits resistance to a wide variety of antimicrobial agents and expresses diverse molecular epidemiology to various established classes of antibiotics including β -lactams, fluoroquinolones, tetracycline and aminoglycosides. Despite the low permeability, hydrophilicity and nonspecific behavior of the outer membrane to small molecular transport, it is inadequate to explain the degree of resistance in *P. aeruginosa*. The resistance mechanism of *P. aeruginosa* against various chemical agents is due to the complex chromosomally encoded genes. Different strains of *P. aeruginosa* having the inherent capacity for biofilm formation, further boosts the resistance under various environmental factors. This chapter explains pathogenicity, mode and types of resistance of *P. aeruginosa*, its impact on the economy and available remediation/reduction measures and treatments.

Keywords: *Pseudomonas aeruginosa*, quorum sensing, adaptive resistance, acquired resistance, intrinsic resistance, efflux system

1. Introduction

Pseudomonas aeruginosa, a Gram-negative pathogen usually found in the hospital, plays a crucial role for nosocomial infection and are also responsible for acute and chronic infection. *P. aeruginosa* is ubiquitous in nature and shows a great susceptibility against various classes of antibiotics [1]. The bacteria get colonize on any surface that contains water and multiply rapidly, carry out all the metabolic functions for growth and development which is an association of complex matrix known as a biofilm [2, 3]. The study predicted that a biofilm makes the bacteria more susceptible in the conditions like antibiotics, exposure in UV light and salinity [4]. Further understanding of the pathogenesis and resistance mechanism is a diverse area of investigation. Due to the complex biofilm forming ability, *Pseudomonas* species shows a great resistivity to various classes of antibiotics which are used to persistently overcome the microbial infection. The occurrence of *Pseudomonas* species in the hospitals helps to form the biofilms on the medical instruments (surface only) and other similar devices along with the implants in the patients [5, 6].

Pseudomonas species are used as a model organism for the study of biochemical mechanisms responsible for the susceptibility of the pathovars against a wide variety of antibiotics groups like amikacin, gentamicin, carbapenem, ofloxacin, ciprofloxacin, tigecycline, tobramycin and norfloxacin [7, 8].

The development of resistance by the pathogenic *Pseudomonas* species devise a major problem in the bacterial diversity by altering the genome sequences and the expression of proteins that ultimately improves the resistance of the pathovars [9, 10]. Various biochemical pathways and channel protein functions are affected due to the resistance of the bacteria [11, 12]. At this alarming stage of the scenario in details studies and prevention measures at an earliest is essential to control the same else in near future it may reach beyond our control. Therefore, the present chapter emphasizes on the infections due to *Pseudomonas aeruginosa*, their mechanism of infection and resistance to various classes of antibiotics.

2. Overview of *Pseudomonas aeruginosa* pathogenesis

The infectious diseases caused by *P. aeruginosa* are sometimes fatal for humans as it is a potential threat to people having less immunity like newborns, diseased persons and veterans. Notably, patients suffering from the diseases like cystic fibrosis, urinary tract infection, burn of the skin, leukemia, HIV-AIDS, diabetes, patients having longer stay in hospital environments and persons having organ transplantation are highly susceptible to *P. aeruginosa*. **Table 1** listed the disease, symptoms and its causes.

Disease caused in humans	Symptoms	Adverse effects on human	References
Bacteremia	Fever, fatigue, chills, joint and muscle pain	Increasing bacterial population in the bloodstream	[13]
Pneumonia, sinusitis	Fever, chills, difficulty in breathing, cough with or without sputum production	Deposition of liquids in the parts of the lungs. Swelling and inflammation of the nasal tract	[14]
Folliculitis	Abscess production in the skin, redness of the skin, draining wounds	Inflammation of the hair follicles by bacteria	[15, 16]
External ear canal Infection (otitis externa)	Ear pain, swelling, itching inside the ear, discharge from the ear, sometimes difficulty in hearing	Frequent showering leads to deposition of water and hence the growth of bacteria takes place at that location	[17, 18]
Corneal inflammation (keratitis)	Redness, pain, swelling, inflammation, pus formation, impaired vision	The bacteria adhere to the lens and other parts of an eye within 24 h of its exposure by its cilia and flagella and forms the biofilm	[19, 20]
Urinary tract infection	Burning with urination, cloudy or bloody urine, strong odor, rectal pain (in male), pelvic pain (in female)	The transfer of bacteria into the urethra	[21]
Diabetic foot	Swelling of foot and ankle, dry cracks in the skin (around the heel), corns or calluses	Tissue damage in the foot and severe pain due to ingrown toenails	[22]

Table 1.
Diseases and symptoms of Pseudomonas aeruginosa infection.

SI no.	Strains of <i>Pseudomonas aeruginosa</i>	Showing resistance to antibiotic class	Mode of action	References
1.	PA40, PA43	Amikacin	Multi-drug-resistance (MDR)	[24, 25]
2.	ATCC 27853, P2284	Ticarcillin/clavulanate	Production of β -lactamase	[26]
3.	K385	Chloramphenicol and norfloxacin	Overexpression of <i>mexC-MexD-OprJ</i> operon	[27]
4.	PA-M4	Ciprofloxacin	Overexpression of <i>MexEF-OprN</i> operon	[28]
5.	OCR1	Gentamicin	Overexpression of <i>MexAB-OprM</i> operon	[28]
6.	PAO4222	Carbapenem (imipenem and meropenem)	Loss of porin channels in the outer membrane, expression of OprD and secreting carbapenem-hydrolyzing metalloenzyme	[29]
7.	PAO4098E	Carbenicillin and tobramycin	Inactivation of aminoglycosides enzyme, ribosomal methyl group transferase enzyme	[27]
8.	PAO1	Tigecycline	Inhibition of <i>MexXY-OprM</i> activity	[30]
9.	KG3002	Ofloxacin	Inactivation of <i>MexC</i> operon	[31]
10.	KG3000	Ciprofloxacin	Expression of <i>MexC-MexD-OprJ</i> operon	[32]
11.	PAO1	Fluroquinolones	DNA gyrase topoisomerase IV activity	[33, 34]
12.	PA1109	Polymyxin E (colistin)	Modification in the LPS layer	[35, 36]
13.	PA124	Tetracyclines	Activation of <i>MexXY-OprM</i> efflux pump	[37]
14.	PAO1	Quinolones	Expression of <i>MexEF-OprN</i> efflux pump due to mutation of NfxB, NfxC and NalB	[38, 39]
15.	ATTC 27853, K1178	Cephalosporin	Overexpression of <i>MexAB-OprM</i> efflux pump due to the NalB mutation	[40]

Table 2.
Antibiotics resistance in different strains of Pseudomonas aeruginosa.

The resistance of *P. aeruginosa* to different aminoglycoside agents show a tremendous threat to public health as well as constrains the therapeutic choice available. The use of multiple drugs against the diseases in a low dose make the *P. aeruginosa* strains more resistant to a wide range of antibiotics [23]. The different strains of *P. aeruginosa* showing resistance to various antibiotic classes along with the pathway of resistant have been demonstrated in **Table 2**.

3. Pathogenicity of *Pseudomonas aeruginosa*

The virulence property of *P. aeruginosa* is mainly due to the presence of factors like alkaline protease, elastase, pyoverdin, pyocyanin, exotoxins and cytotoxins.

This virulence factors are commonly restricted to immunocompromised patients. The pathovars also produces a kind of exopolysaccharide known as alginate in patients having chronic respiratory infections. These alginate serves as the adhesive on the solid surfaces and also protects the bacteria from unfavorable environmental conditions [41]. The bacteria also produce alginate lyase enzyme which can cleave the polysaccharide into short oligosaccharide units it has been observed that both the biosynthesis and degradation process plays a vital role in the infection process [42, 43]. Presence of extracellular virulence factors and cell surface associated structures promotes its pathogenicity [44, 45].

P. aeruginosa binds to the ganglioside present in the host epithelial surface with the help of lipopolysaccharide and bacterial adhesins (i.e. type-IV pili and flagella). Type-IV also facilitates the bacterial movement along the host cell surface known as “twitching motility” which enhances the development of biofilm [46]. After the attachment to the host cell type III secretion system (T3SS) get activated and makes pore or a channel (i.e. translocon) on the cell membrane by injecting cytotoxic effector proteins into the cytosol of host cell [47, 48]. Mainly four different types of toxins are found in the *P. aeruginosa* sp. i.e. Exoenzymes S, T, U and Y. EXoS, ExoT and ExoU are responsible for N-terminal GTPase-activating proteinase (GAP) activity, C-terminal ADP-ribosyltransferase activity (ADPRT) and adenylate cyclase activity respectively [49]. It has been found that the ExoU is also a potent cytotoxin to cleave the host membrane phospholipid layers i.e. Phospholipase A2 (PLA2) activity. The ExoU initiates the inflammation by secreting the arachidonic acid for activating lipoxygenase and cyclooxygenase pathways and results the production of prostaglandins. *P. aeruginosa* secretes an Exotoxin A which is a type of ADPRT that causes cell death by inhibiting protein synthesis due to suppression of host elongation factor 2 (EF2) [50]. The lipase and phospholipase of the bacteria dissolve the surfactant lipids and phospholipids of the host cell membranes. The blue-green pigment pyocyanin develops the oxidative stress in host cells by disrupting the host catalase and electron transport system (ETS) hence suppresses the phagocytosis activity of the host immune system [51].

The type-VI secretion system (T6SS) seen in case of *P. aeruginosa* facilitates the interaction of this pathogen with other organism and provides defence from other bacteria. The H1-, H2- and H3-T6SS are the three distinct T6SS observed in this pathovars. The H1-T6SS is being used for the physiological study of antimicrobial activity [52, 53]. The H2- and H3-T6SS plays dual role in the interaction with both prokaryotic and eukaryotic cell. The production of proteases degrades the covered mucin and complement systems which results the disruption of the tight junctions between the host epithelial cells. Then the bacteria spreads from one cell to others by secreting the phospholipase by damaging the cell membrane [54]. The release of pyocyanin and pyoverdine interfere with the electron transport pathways and redox cycling system of the host cells. LasA and LasB are the two types of elastases produced by *P. aeruginosa*, commonly responsible for the burn wound infection and acute lung infections. The LasA hydrolyze the penta-glycine bridge necessary for the stabilization of the peptidoglycan in the cell wall and the LasB is responsible for the opsonisation of the lung surfactant proteins A and D [55].

4. Resistance for antimicrobials in *Pseudomonas aeruginosa*

A wide group of *P. aeruginosa* strains are resistance to various classes of antibiotics or antibacterial agents that makes it difficult to control the infection. The resistance in *Pseudomonas* species is broadly due to the below detail explained methods studied previously. **Figure 1** explicitly elaborate on various mechanism of

Figure 1.
 Resistance of *P. aeruginosa* to various antimicrobials as (1) shows the enzymatic modification, (2) impermeability resistance, (3) efflux system and (4) modification in the outer membrane.

P. aeruginosa resistance. The resistance pattern and mechanism behind the development of resistance in the *Pseudomonas* species are the topic of interest for the researchers as it will help to develop the polyphylactic procedures and mitigation of infection due to *P. aeruginosa*.

4.1 Enzymatic modification

P. aeruginosa consists of elements generally termed as transposons which induce resistance due to the modification of aminoglycoside enzymes. The infection due to the pathogen is usually combated by various class/groups of aminoglycoside antibiotics like kanamycin, gentamicin, streptomycin, amikacin and neomycin. Previous studies elucidate that, there are three types of enzymatic conformational change which are accountable for the resistance against the bactericidal compounds. These are phosphorylation of aminoglycoside phosphoryl transferase (APH) [56, 57] adenylation of aminoglycoside nucleotidyl transferase (ANT) and acetylation of aminoglycoside acetyl transferase (AAC) [58, 59].

The conformational modification and phosphorylation in the 3'-OH group is carried out by the APH enzyme. APH (3') family of enzymes shows resistance against streptomycin, butirocin, amikacin, kanamycin and neomycin by encoding the genes such as *aphA* and *hpaA* which are involved in the metabolism of 4-hydroxy-phenylacetic acid (4-HPA). However, APH (2'') shows resistance to tobramycin and gentamicin classes of antibiotics. Due to adenylation of ANT enzymes *P. aeruginosa* increases resistance towards tobramycin, gentamicin, streptomycin, isepamicin and amikacin [60, 61]. The family of enzymes such as ANT (2''), (3'') and (4') also shows a similar type of resistance in different strains of *P. aeruginosa* isolated from hospitals and intensive care unit (ICU) premises [62]. The N-terminal positions (1, 2', 3 and 6') of the (AAC) shows the enzymatic acetylation. Amongst various families, AAC (3-I), (3-II) and (3-III) are also resistant to gentamicin, tobramycin and kanamycin antibiotics respectively [63]. Apart from that AAC (6') family of enzymes contributes to the resistance along with akamicin [64].

4.2 Impermeability resistance

Impermeability to various exocompounds in Gram-negative bacteria is due to lipopolysaccharide (LPS) present in the cell wall. LPS is made up of lipid A, oligosaccharide core and O antigen regions which are linked covalently [65]. The lipid A region is hydrophobic in nature and made up of a disaccharide of glucosamine which is phosphorylated and helps in the anchoring of LPS to the cell membrane. The core oligosaccharide is accumulation of sugar, ethanolamine, phosphate and amino acids and can be divided into inner and outer core. The O antigen is the outer domain of bacterial LPS made up of repeating glycan polymers and attached with the core region. It has been observed that the deletion of lipid A makes the bacteria susceptible to various classes of hydrophobic antibiotics and degradation of O side chains determine the smoothness and roughness of the LPS [66, 67]. The use of ethylenediaminetetraacetic acid (EDTA), some organic acids like lactic acid and citric acid are found to alter the impermeability of the *Pseudomonas* species. These chelating agents can neutralize the negatively charged oligosaccharide core by binding with the (Mg^{2+}) cations in the LPS molecule and promotes the removal of LPS molecules [68]. The accumulation of aminoglycoside level decreases in the case of *P. aeruginosa* leading to low uptake and hence shows impermeability resistance which has been reported in the strains isolated from the cystic fibrosis patients [58]. Similarly, tobramycin resistance due to impermeability was seen when studied for endocarditis in case of rabbits.

4.3 Through the efflux system

The drug efflux system in bacteria includes three major components i.e. outer membrane channel-forming protein (OMF), resistance nodulation division (RND) which helps in drug-protein antiport process and the membrane fusion protein that acts as a periplasmic link between above two components [69]. The *mexXY* operon codes the inner membrane protein (i.e. *MexY*) and periplasmic protein (i.e. *MexX*). Resistance nodulation division (RND) involves the *MexXY* efflux system which develops the resistance in *Pseudomonas* species [70, 71]. *MexAB-OprM* shows resistance against ticarcillin, broad-spectrum cephalosporin and β -lactam of clinical isolates, while the combination of *MexAB-OprM*, *MexCD-OprJ* and *MexXY-OprM* shows the carbapenem resistance [72]. The bacterial isolates like *Burkholderia pseudomallei* and *Escherichia coli* involve the three component systems known as RND type aminoglycoside efflux system. Treatment with ofloxacin and gentamicin increases the level of *MexXY* expression in case of mutants compared to wild-type strains [73, 74]. The wild-type of strains of *Pseudomonas* is resistance to the antibiotic classes like tetracyclines, aminoglycosides, glycolcyclines and erythromycin but the *MexXY* can express in presence of diverse class of antibiotics like lincomycin [75], macrolides [76], fluoroquinolones [77], chloramphenicol [30], β -lactams [72], novobiocin [78] along with the wild type of antibiotic classes. In the reduced aminoglycosides condition both adaptive and impermeability resistance in the *Pseudomonas* sp. is expressed. The expression of *MexXY* gene is regulated by *mexZ* repressor, present in the upstream region of *MexXY* region of the gene and belongs to tetracycline repressor protein (TetR) and AcrR repressor protein family [79].

4.4 Modification in the outer membrane

The exoskeleton of the Gram-negative bacteria is present to resist against the adverse environmental conditions. Likewise, the outer membrane of *P. aeruginosa* is designed in such a way that it can permit small hydrophilic molecules and inhibit

larger molecules such as antibiotics [80]. Due to the crucial arrangement of aquaporin proteins in the cell membrane, the small hydrophilic antibiotics of quinolone and β -lactam classes can pass through the outer membrane. *P. aeruginosa* strains produce four major aquaporins (i.e. oprP, oprD, oprF and oprB) and two minor aquaporins (i.e. oprC, oprE) whereas the mutant strains lack oprF [81, 82]. The oprD is a specialized porin molecule present in bacterial membrane that helps in the process of up-taking positively charged amino acids like arginine and lysine [83]. The minimum inhibitory concentration increases due to the loss of oprD porin from the outer membrane of the *Pseudomonas* sp. thus increasing the resistance to imipenem class of antibiotics [84]. As the porin channels are impermeable to the polymyxin E and aminoglycoside, these molecules bind with the LPS present in the outer membrane, destructs the barrier and allows the antibiotics to enter into the bacterial cells [85]. Through this mechanism the aminoglycosides can enter into the cytoplasm of the bacterial cell and disturb the protein synthesis process in the ribosomes that kills the bacteria simultaneously. But the overexpression of the oprH an outer membrane protein [86], prevents the binding of antibiotics to LPS making it resistant for laboratory strains of *Pseudomonas* species.

4.5 Resistance by biofilm

Bacterial communities aggregate themselves to a substratum and encapsulated in a proteinous polysaccharide of matrix evolved during adverse environmental condition such as various irradiation treatments and therapy which is known as biofilm. Mostly these polysaccharide/polymeric matrix leads to the formation of biofilms over a water surface and shows resistance and enhances their survivability against the antimicrobial agents [87, 88]. The formation of biofilm is predominantly found in case of various biomedical instruments such as catheter, implants, ventilator and dialyser used patients residing in the hospital [89]. The bacteria are found to evade from host immune response due to the formation of biofilms and helps in promoting collateral damage to the tissues. Only few antibiotic classes act as an effective bactericidal agent for the free-floating bacteria but it fails to act against the bacteria forming biofilms as the biofilms are 1000 times more invulnerable to it [90, 91]. During environmental stress conditions, the bacteria change from free-living unicellular form to the planktonic form and then to the attached biofilm structure which enables the survivability of the bacteria. The matured biofilm starts to segregate from a place and develop an immobile structure in the new surfaces for colonization [92, 93]. The chemical therapy of antibiotics was not effective as the molecules cannot penetrate into the complex biofilm matrix due to the production of cover like exopolysaccharides matrix known as glycocalyx [94, 95]. Mostly the pathovars of *P. aeruginosa* forms the biofilm in the dialysis membrane and restricts the diffusion of piperacillin antibiotic into the complex aggregation [96]. It is pertinent to mention here that the bacterial biofilm is resistant to various classes/groups of antibiotics.

4.6 Resistance by quorum sensing

The *P. aeruginosa* has been found to be resistive to various bactericidal agents and mainly infects to the people suffering from HIV-AIDS and cancer due to the compromised immune system, use of broad-spectrum antibiotics for a longer duration and dependency on life support medical devices like a catheter, ventilator and dialyser. The bacteria communicate with each other by secreting extracellular signaling molecules known as autoinducer. The autoinducer level is directly proportional to the growth of bacterial population, hence with the increase in bacterial population the

accumulation of autoinducer in the environment is at the peak [10, 97]. This process of production, release of signaling molecules is termed as quorum sensing.

There are four types of quorum sensing pathways discovered for the *P. aeruginosa* species which includes the LasR and LasI, RhlR and RhlI, PqsR-quinolone controlled system and the integrated quorum sensing (IQS) system which works under limiting conditions of phosphate [98, 99]. The formation of complexes of LasR with 3-oxo-C12-HSL activates the LasI synthase gene which helps in the process of autoinduction. The LasR complex regulates the expression of rhlI and rhlR genes along with the PQS systems which are related to the second and third mode of quorum sensing system of pathway respectively. The activation of its own regulon by the binding of C4-HSL with RhlR induces the second induction processes. The activation of RhlR is induced by PqsR-PQS complex which regulates the three modes of signaling in Quorum sensing along with inhibits the expression of the pqsR and pqsABCD. The ratio of 3-oxo-C12-HSL to C4-HSL gives an idea about the activation of PQS [100, 101]. The virulence property of *P. aeruginosa* is controlled by the RhlR along with C4-HSL and PqsR or LasR. Incase of the isolates of *P. aeruginosa* from the cystic fibrosis patients the mutations in the LasR supplies the autoinducer as there is the necessity of phosphate starvation protein (PhoB). This LasR activates the expression of pqs genes by the production of IQS which expresses the rhl gene hence shows the pathogenicity [102].

4.7 Others

Pseudomonas species also include the resistance mechanism like adaptive resistance, acquired resistance and intrinsic resistance which further helps in the increasing the resistivity of the pathogen to a wide range of antibiotic class.

4.7.1 Adaptive resistance

The resistance which is dependent on the physical and chemical stresses, growth states and promotes the initiation of the regular processes inside the cell in the presence of antibiotics and reverts back to the primary condition in the removal of the inducers are known as adaptive resistance [103, 104]. Previous research studies manifested that the resistance is due to many factors like the use of sub-inhibitory concentration of antimicrobial agents, polyamines, heat shock, SOS response, pH imbalance and anaerobiosis condition [105, 106]. *P. aeruginosa* was found to develop adaptive resistance against divalent Ca^{2+} and Mg^{2+} ions and the polymyxins which are controlled by *PmrAB* and *PhoPQ* pathways [107]. *P. aeruginosa* gradually reduces susceptibility in the presence of antibiotics and is altered in absentia this phenomena are reversible in nature and scientifically termed as the adaptive resistance [108]. The extensive studies revealed that adaptive resistance can also be developed in both *in vivo* and *in vitro* conditions due to the administration of antibiotics into the bacterial culture for few hours and this resistance disappears after the removal of antibiotics from the media [109]. But it is observed that the organism shows resistance when there is a low accumulation of the aminoglycosides. The resistance induced through drug efflux system and due to the gene expression associated with anaerobic respiration. The bacteria were grown in the anaerobic condition and nitrate environment to check the accumulation and the uptake of aminoglycoside and found that *P. aeruginosa* is capable of showing resistance in the anaerobic conditions [110].

4.7.2 Acquired resistance

The acquired resistance involves the transfer of plasmids, prophages, DNA elements and transposons by means of transduction, transformation and

conjugation. This horizontal transfer shows the β -lactam and aminoglycoside resistance in *P. aeruginosa* [111]. The chemical modification of the aminoglycosides alters the affinity of a 30S subunit of ribose sugar to the target. Antibiotic drugs like cephalosporin, carbapenem [112] and penicillin [113] help in the process of development of resistance property in case to *P. aeruginosa* [112]. The mutational resistance occurred due to the formation of biofilms and the action of DNA-damaging agents. The mutation frequency is found to be increased by 10-fold, greater than 100-fold and 70-fold if the resistance is caused by meropenem [85], ciprofloxacin and if any mutation in genes respectively [114]. The downregulation of antioxidant enzymes damages the DNA in the biofilms. The library screening of cystic fibrosis (CF) patients describe that there are various mutators play a significant role during the early infection stages, *mutL* and *mutS* are the hypermutators which are widely found. The mutation in genes *mexR*, *mexZ* and *nfxB* is due to the overexpression of MexAB-OprM, MexXY-OprM and MexCD-OprJ efflux pump respectively. OprD is a porin that suppresses the uptake of imipenem [115] and another antibiotic [116] leading to the clinical resistance. The *ampC* β -lactamase, *AmpD* mutate and controls the activity of *AmpR* regulator [117]. The *P. aeruginosa* clinical strain shows resistance to mutations in gyrase (*gyrA*) and *gyrB* as well as *parC* and *parE*. Overlay we can demonstrate that the mutations in the unrelated genes give rise to acquired resistance against different antibiotics.

4.7.3 Intrinsic resistance

The intrinsic resistance is due to the combination of the efflux system along with the β -lactamase and the low outer membrane permeability, the entry of antibiotic molecules through the outer membrane of the bacteria [8]. The increase in antibiotic concentration in the environment helps in the low permeability of the outer membrane permits the entry of larger compounds and antibiotics into the cell with the help of porin protein channels and makes the bacteria resistant this slow process helps in increased resistance of the organism [83, 118]. The intrinsic resistance is carried out by the help of multi-drug efflux systems like MexAB-OprM and MexXY-OprM operon along with the inactivation of enzyme β -lactams by hydrolysis [119, 120].

5. Impact of *Pseudomonas aeruginosa* on the economy

The low membrane permeability, overexpression of efflux pump and deletion of porin channels are the cause behind the resistance of *Pseudomonas* species. *P. aeruginosa* was predominantly found in the ICUs of European continents hence put in the list of “ESKAPE” pathogens by the Infectious Disease Society of America [121, 122]. The existing antibacterial agents are not effective against these isolates and hence a severe threat for public health. A study in China for the bacterial resistance surveillance demonstrated that the resistance in case of hospital-acquired infection (HAI) is prevalence than community-acquired infection (CAI) [123]. Relatively few studies explained about the outbreak of Multi-drug resistance (MDR) in *P. aeruginosa* species. The worldwide study of *Pseudomonas* infections gives us the idea that in the year 2002 14% and in 2003 9.9% resistance were found in ICU isolates and nosocomial infections in United states [77]. During 1997–1999 8.2% and 4.7% of resistance were due to nosocomial infections in South America and Europe respectively [124, 125]. In 2001 2.8% and in 2005 6.9% of resistance were due to nosocomial infections in Japan [126] and Malaysia [127].

The National Nosocomial Infection Surveillance System (NNIS) also conducted the study for statistical analysis of the resistance developed by the hospital strains of *P. aeruginosa* and define that the hospital samples are more resistive to various groups of antibiotic classes [128]. The resistance to various classes of antibiotic by *P. aeruginosa* is a new threat to our defence system as once compromised it will be a difficult task to control the spread and infection of the bacteria among the living system. It has been also reported that the bacteraemia was not in control by the administration of antibiotics as it was spread by the antibiotic-resistant strains of *P. aeruginosa* [129].

Due to hospitalization for a significant period of time in the ICU [130] of a patient suffering from respiratory disorder [110], kidney disease [89] and other diseases which needs the ventilator along with the medical device installation are more prone to the infection of *P. aeruginosa* [131]. The administration of various drugs makes the *Pseudomonas* strain more resistive due to mechanisms like multi-drug-resistance (MDR), efflux systems, and loss of porin proteins from the outer membrane. Extensive research work is necessary to understand the infection mechanism and the development of resistance in the bacteria, the suitable combination of antibiotic molecules which will overcome the resistant behaviour and eradication of the bacterial biofilm without affecting the other processes in the living beings.

6. Mitigation of resistance

The eradication of the resistance is highly necessary for the prevention followed by cure to *Pseudomonas* infection for healthy sustenance. So, research is still going on to overcome the resistance by the organism and combinational therapeutic approach is found to be an effective tool against the resistance of the *Pseudomonas* species.

Cross-infection through hospital personnel gives rise to 30–40% of infection so irrespective of cost and time use of masks, cloths, gloves, antiseptics for the proper isolation can minimize the resistant developed in the pathovars [132]. It was observed that usual laboratory methods failed to detect the Antimicrobial-Drug resistance hence new testing methods, standards and guidelines implemented by various national and international clinical research groups for the early detection and control its outbreak [133]. The synergistic of two or more anti-bactericidal molecules is found to be an effective than monotherapy to overcome the resistance. The combination of polymixin with tobramycin is found to be an effective antimicrobial for inhibition in the formation of biofilms [134]. The combinational administration of tobramycin with aminoglycoside and macrolide clarithromycin shows a devastating effect against the biofilm [79]. Likewise, the integration of azithromycin with the tobramycin helped to destroy the bacterial biofilm when treated with *in vitro* condition [135].

The use of nitric oxide (NO) was reported to trigger the downstream of signal processing in quorum sensing and hence the production of cyclic-di-GMP decreases hence the extracellular matrix of biofilm get destroyed [136]. The introduction of deoxyribonuclease (DNase) directly into the biofilm of the bacterial colony as it digests the environmental DNA (eDNA) enzymatically. The *P. aeruginosa* contains a molecule known as acyl-homoserine lactones (AHL), the blockage of signaling of this molecule prevents the formation of biofilms [137]. The *rsaL* gene expression acts as a negative regulator of the *lasI* gene expression which is responsible for the quorum sensing in the strains of *P. aeruginosa* [138]. The *PmrAB* and *PhoPQ* can alter the permeability of the outer membrane as the level of divalent ions decrease it increase the extracellular DNA in the biofilms and shows resistance to cationic bactericidal peptides and polymyxins [139]. Due to this phenomenon, the addition of amino

arabinose to the 1st and 4th phosphate position in lipid A of the LPS and the net negative charge neutralized and the cations can enter into the bacterial cell [140].

The medical equipment and the biomaterial use for implantation purpose are coated with silver which reduces the adherence and biofilm producing ability of the bacteria. The novel compounds like curlicides and pilicides have been reported to inhibit the role of adhesin molecules and hence reduces the formation of biofilms on the surfaces. The use of nanomaterials of graphene and zinc as the coating of biomedical implants are found to be effective against the biofilm formation [141]. In some instances, it is necessary to replace the device after prolonged use with the patient/s. The small molecular artificially engineered peptide 1018 was discovered with the anti-biofilm activity [142].

The pharmaceutical industries are working towards the development of vaccines to tackle the antimicrobial resistance and few are under clinical trials which are believed to be effective against the resistance [143, 144]. There are several vaccines such as polysaccharide-protein conjugates, LPS-O antigen, OprI and OprF membrane protein, live-attenuated, flagella and DNA vaccines are known to be invented for the control of antimicrobial resistance of *P. aeruginosa*. But the recombinant vaccine IC43, OprI and OprF and flagella vaccines are found effective and are under clinical trials for cystic fibrosis patients [145]. Apart from the above various NGOs and educational groups are playing a great role to educate the students, doctors, hospital personnel and society by making people aware about the use of proper dose and medicines by consulting the physician along with the maintenance of hygiene in the surroundings.

7. Concluding remarks

P. aeruginosa as an emerging human pathogen causes an array of diseases in immunocompromised patients, newborns as well as healthy persons. The infection as a biofilm is much more severe than monoculture. Various antimicrobial/antibiotics treatment leads to not only increases the resistance in different strains of *P. aeruginosa* but also increase the disease incidence. The present chapter clearly enlightens various mechanisms of infection of *P. aeruginosa*, its biofilms and resistance pathways/mechanisms, global impact due to infections which further paves the way for various remediation in future through improved implementations of genetic engineering and advances nanotechnology tools.

Author details

Swaraj Mohanty, Bighneswar Baliyarsingh and Suraja Kumar Nayak*
Department of Biotechnology, College of Engineering and Technology (CET),
Biju Patnaik University of Technology (BPUT), Bhubaneswar, Odisha, India

*Address all correspondence to: surajnayak3@gmail.com

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Kerckhoffs AP, Ben-Amor K, Samsom M, van der Rest ME, de Vogel J, Knol J, et al. Molecular analysis of faecal and duodenal samples reveals significantly higher prevalence and numbers of *Pseudomonas aeruginosa* in irritable bowel syndrome. *Journal of Medical Microbiology*. 2011;**60**(2):236-245
- [2] Murga R, Miller J, Donlan R. Biofilm formation by gram-negative bacteria on central venous catheter connectors: Effect of conditioning films in a laboratory model. *Journal of Clinical Microbiology*. 2001;**39**(6):2294-2297
- [3] Stickler D. Susceptibility of antibiotic-resistant Gram-negative bacteria to biocides: A perspective from the study of catheter biofilms. *Journal of Applied Microbiology*. 2002;**92**:163S-170S
- [4] Johani K, Abualsaud D, Costa DM, Hu H, Whiteley G, Deva A, et al. Characterization of microbial community composition, antimicrobial resistance and biofilm on intensive care surfaces. *Journal of Infection and Public Health*. 2018;**11**(3):418-424
- [5] De Silva B, Wimalasena S, Hossain S, Pathirana H, Heo G-J. Characterization of quinolone resistance of *Pseudomonas aeruginosa* isolated from pet chinese stripe-necked turtles (*Ocadia sinensis*). *Asian Journal of Animal and Veterinary Advances*. 2017;**12**(3):152-160
- [6] Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. *New England Journal of Medicine*. 2010;**362**(19):1804-1813
- [7] Sriramulu DD, Lünsdorf H, Lam JS, Römling U. Microcolony formation: A novel biofilm model of *Pseudomonas aeruginosa* for the cystic fibrosis lung. *Journal of Medical Microbiology*. 2005;**54**(7):667-676
- [8] Mesaros N, Nordmann P, Plésiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, et al. *Pseudomonas aeruginosa*: Resistance and therapeutic options at the turn of the new millennium. *Clinical Microbiology and Infection*. 2007;**13**(6):560-578
- [9] Overhage J, Bains M, Brazas MD, Hancock RE. Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. *Journal of Bacteriology*. 2008;**190**(8):2671-2679
- [10] Smith RS, Iglewski BH. *P. aeruginosa* quorum-sensing systems and virulence. *Current Opinion in Microbiology*. 2003;**6**(1):56-60
- [11] Tenover FC. Mechanisms of antimicrobial resistance in bacteria. *American Journal of Infection Control*. 2006;**119**(6):S3-S10
- [12] Normark BH, Normark S. Evolution and spread of antibiotic resistance. *Journal of Internal Medicine*. 2002;**252**(2):91-106
- [13] Tam VH, Rogers CA, Chang K-T, Weston JS, Caeiro J-P, Garey KW. Impact of multidrug-resistant *Pseudomonas aeruginosa* bacteremia on patient outcomes. *Antimicrobial Agents and Chemotherapy*. 2010;**54**(9):3717-3722
- [14] Miko BA, Pereira MR, Safdar A. Respiratory tract infections: Sinusitis, bronchitis, and pneumonia. In: *Principles and Practice of Transplant Infectious Diseases*. New York, NY: Springer; 2019. pp. 339-349
- [15] Moore LS, Cunningham J, Donaldson H. A clinical approach to managing *Pseudomonas aeruginosa* infections. *British Journal of Hospital Medicine*. 2016;**77**(4):C50-CC4

- [16] Olszewski AE, Karandikar MV, Surana NK. Aeromonas as a cause of purulent folliculitis: A case report and review of the literature. Journal of the Pediatric Infectious Diseases Society. 2017;**6**(1):e1-e3
- [17] Aliyu I, Kumurya A, Bala J, John O. Bacteriology of otitis media and its host-environmental-infection factors. Asia Pacific Environmental and Occupational Health Journal. 2017;**3**(1):20-27
- [18] Heward E, Cullen M, Hobson J. Microbiology and antimicrobial susceptibility of otitis externa: A changing pattern of antimicrobial resistance. The Journal of Laryngology & Otology. 2018;**132**(4):314-317
- [19] Saraswathi P, Beuerman RW. Corneal biofilms: From planktonic to microcolony formation in an experimental keratitis infection with *Pseudomonas aeruginosa*. The Ocular Surface. 2015;**13**(4):331-345
- [20] Kugadas A, Christiansen SH, Sankaranarayanan S, Surana NK, Gauguet S, Kunz R, et al. Impact of microbiota on resistance to ocular *Pseudomonas aeruginosa*-induced keratitis. PLoS Pathogens. 2016;**12**(9):e1005855
- [21] Cole SJ, Records AR, Orr MW, Linden SB, Lee VT. Catheter-associated urinary tract infection by *Pseudomonas aeruginosa* is mediated by exopolysaccharide independent biofilms. Infection and Immunity. 2014;**82**(5):2048-2058
- [22] Giordano P, Song J, Pertel P, Herrington J, Kowalsky S. Sequential intravenous/oral moxifloxacin versus intravenous piperacillin-tazobactam followed by oral amoxicillin-clavulanate for the treatment of complicated skin and skin structure infection. International Journal of Antimicrobial Agents. 2005;**26**(5):357-365
- [23] Smith DJ, Ramsay KA, Yerkovich ST, Reid DW, Wainwright CE, Grimwood K, et al. *Pseudomonas aeruginosa* antibiotic resistance in A ustralian cystic fibrosis centres. Respiriology. 2016;**21**(2):329-337
- [24] Falagas ME, Koletsi PK, Bliziotis IA. The diversity of definitions of multidrug-resistant (MDR) and pandrug-resistant (PDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Journal of Medical Microbiology. 2006;**55**(12):1619-1629
- [25] Torres C, Perlin MH, Baquero F, Lerner DL, Lerner SA. High-level amikacin resistance in *Pseudomonas aeruginosa* associated with a 3'-phosphotransferase with high affinity for amikacin. International Journal of Antimicrobial Agents. 2000;**15**(4):257-263
- [26] Lin D, Foley S, Qi Y, Han J, Ji C, Li R, et al. Characterization of antimicrobial resistance of *Pseudomonas aeruginosa* isolated from canine infections. Journal of Applied Microbiology. 2012;**113**(1):16-23
- [27] Cézard C, Farvacques N, Sonnet P. Chemistry and biology of pyoverdines, *Pseudomonas* primary siderophores. Current Medicinal Chemistry. 2015;**22**(2):165-186
- [28] De Kievit TR, Parkins MD, Gillis RJ, Srikumar R, Ceri H, Poole K, et al. Multidrug efflux pumps: Expression patterns and contribution to antibiotic resistance in *Pseudomonas aeruginosa* biofilms. Antimicrobial Agents and Chemotherapy. 2001;**45**(6):1761-1770
- [29] Imamovic L, Ellabaan MMH, Machado AMD, Citterio L, Wulff T, Molin S, et al. Drug-driven phenotypic convergence supports rational treatment strategies of chronic infections. Cell. 2018;**172**(1-2):121-134. e14
- [30] Dean CR, Visalli MA, Projan SJ, Sum P-E, Bradford PA. Efflux-mediated

- resistance to tigecycline (GAR-936) in *Pseudomonas aeruginosa* PAO1. Antimicrobial Agents and Chemotherapy. 2003;47(3):972-978
- [31] Lomovskaya O, Warren MS, Lee A, Galazzo J, Fronko R, Lee M, et al. Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: Novel agents for combination therapy. Antimicrobial Agents and Chemotherapy. 2001;45(1):105-116
- [32] Piddock LJ. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clinical Microbiology Reviews. 2006;19(2):382-402
- [33] Muller C, Plésiat P, Jeannot K. A two-component regulatory system interconnects resistance to polymyxins, aminoglycosides, fluoroquinolones, and β -lactams in *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy. 2011;55(3):1211-1221
- [34] Nouri R, Ahangarzadeh Rezaee M, Hasani A, Aghazadeh M, Asgharzadeh M. The role of gyrA and parC mutations in fluoroquinolones-resistant *Pseudomonas aeruginosa* isolates from Iran. Brazilian Journal of Microbiology. 2016;47(4):925-930
- [35] Olaitan AO, Morand S, Rolain J-M. Mechanisms of polymyxin resistance: Acquired and intrinsic resistance in bacteria. Frontiers in Microbiology. 2014;5:643
- [36] Moskowitz SM, Brannon MK, Dasgupta N, Pier M, Sgambati N, Miller AK, et al. PmrB mutations promote polymyxin resistance of *Pseudomonas aeruginosa* isolated from colistin-treated cystic fibrosis patients. Antimicrobial Agents and Chemotherapy. 2012;56(2):1019-1030
- [37] Mawabo IK, Noumedem JA, Kuiate JR, Kuete V. Tetracycline improved the efficiency of other antimicrobials against gram-negative multidrug-resistant bacteria. Journal of Infection and Public Health. 2015;8(3):226-233
- [38] Nejma MB, Sioud O, Mastouri M. Quinolone-resistant clinical strains of *Pseudomonas aeruginosa* isolated from University Hospital in Tunisia. 3 Biotech. 2018;8(1):1
- [39] Jacoby GA. Plasmid-mediated quinolone resistance. In: Antimicrobial Drug Resistance. Berlin: Springer; 2017. pp. 265-268
- [40] Barnes MD, Taracila MA, Rutter JD, Bethel CR, Galdadas I, Hujer AM, et al. Deciphering the evolution of cephalosporin resistance to ceftolozane-tazobactam in *Pseudomonas aeruginosa*. MBio. 2018;9(6):e02085-e02018
- [41] Streeter K, Katouli M. *Pseudomonas aeruginosa*: A review of their pathogenesis and prevalence in clinical settings and the environment. Infection, Epidemiology and Microbiology. 2016;2(1):25-32
- [42] Gellatly SL, Hancock RE. *Pseudomonas aeruginosa*: New insights into pathogenesis and host defenses. Pathogens and Disease. 2013;67(3):159-173
- [43] Alhazmi A. *Pseudomonas aeruginosa*-pathogenesis and pathogenic mechanisms. International Journal of Biology. 2015;7(2):44
- [44] De Bentzmann S, Plésiat P. The *Pseudomonas aeruginosa* opportunistic pathogen and human infections. Environmental Microbiology. 2011;13(7):1655-1665
- [45] Kipnis E, Sawa T, Wiener-Kronish J. Targeting mechanisms of *Pseudomonas aeruginosa* pathogenesis. Médecine et Maladies Infectieuses. 2006;36(2):78-91

- [46] Sousa A, Pereira M. *Pseudomonas aeruginosa* diversification during infection development in cystic fibrosis lungs—A review. *Pathogens*. 2014;**3**(3):680-703
- [47] Azam MW, Khan AU. Updates on the pathogenicity status of *Pseudomonas aeruginosa*. *Drug Discovery Today*. 2019;**24**(1):350-359
- [48] Feltman H, Schulert G, Khan S, Jain M, Peterson L, Hauser AR. Prevalence of type III secretion genes in clinical and environmental isolates of *Pseudomonas aeruginosa*. *Microbiology*. 2001;**147**(10):2659-2669
- [49] Krueger KM, Barbieri JT. The family of bacterial ADP-ribosylating exotoxins. *Clinical Microbiology Reviews*. 1995;**8**(1):34-47
- [50] Rüssmann H. Inverted pathogenicity: The use of pathogen-specific molecular mechanisms for prevention or therapy of disease. *International Journal of Medical Microbiology*. 2004;**293**(7-8):565-569
- [51] Hazlett LD. Pathogenic mechanisms of *P. aeruginosa* keratitis: A review of the role of T cells, Langerhans cells, PMN, and cytokines. *DNA and Cell Biology*. 2002;**21**(5-6):383-390
- [52] Moscoso JA, Mikkelsen H, Heeb S, Williams P, Filloux A. The *Pseudomonas aeruginosa* sensor RetS switches Type III and Type VI secretion via c-di-GMP signalling. *Environmental Microbiology*. 2011;**13**(12):3128-3138
- [53] Sana TG, Hachani A, Bucior I, Soscia C, Garvis S, Termine E, et al. The second type VI secretion system of *Pseudomonas aeruginosa* strain PAO1 is regulated by quorum sensing and Fur and modulates internalization in epithelial cells. *Journal of Biological Chemistry*. 2012;**287**(32):27095-27105
- [54] Kim JH, Park E-S, Shim JH, Kim M-N, Moon W-S, Chung K-H, et al. Antimicrobial activity of p-hydroxyphenyl acrylate derivatives. *Journal of Agricultural and Food Chemistry*. 2004;**52**(25):7480-7483
- [55] Hobden JA. *Pseudomonas aeruginosa* proteases and corneal virulence. *DNA and Cell Biology*. 2002;**21**(5-6):391-396
- [56] Wright GD. Bacterial resistance to antibiotics: Enzymatic degradation and modification. *Advanced Drug Delivery Reviews*. 2005;**57**(10):1451-1470
- [57] Strateva T, Yordanov D. *Pseudomonas aeruginosa*—A phenomenon of bacterial resistance. *Journal of Medical Microbiology*. 2009;**58**(9):1133-1148
- [58] Poole K. Aminoglycoside resistance in *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*. 2005;**49**(2):479-487
- [59] Bonomo RA, Szabo D. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. *Clinical Infectious Diseases*. 2006;**43**(Supplement_2):S49-S56
- [60] Kotra LP, Haddad J, Mobashery S. Aminoglycosides: Perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrobial Agents and Chemotherapy*. 2000;**44**(12):3249-3256
- [61] Marvig RL, Sommer LM, Molin S, Johansen HK. Convergent evolution and adaptation of *Pseudomonas aeruginosa* within patients with cystic fibrosis. *Nature Genetics*. 2015;**47**(1):57
- [62] Azucena E, Mobashery S. Aminoglycoside-modifying enzymes: Mechanisms of catalytic processes and inhibition. *Drug Resistance Updates*. 2001;**4**(2):106-117
- [63] Ramirez MS, Tolmasky ME. Aminoglycoside modifying enzymes.

Drug Resistance Updates.
2010;**13**(6):151-171

[64] Ruiz-Martínez L, López-Jiménez L, Fusté E, Vinuesa T, Martínez J, Viñas M. Class 1 integrons in environmental and clinical isolates of *Pseudomonas aeruginosa*. International Journal of Antimicrobial Agents. 2011;**38**(5):398-402

[65] Caroff M, Karibian D. Structure of bacterial lipopolysaccharides. Carbohydrate Research. 2003;**338**(23):2431-2447

[66] Crompton R, Williams H, Ansell D, Campbell L, Holden K, Cruickshank S, et al. Oestrogen promotes healing in a bacterial LPS model of delayed cutaneous wound repair. Laboratory Investigation. 2016;**96**(4):439-449

[67] Zhang L, Dhillon P, Yan H, Farmer S, Hancock RE. Interactions of bacterial cationic peptide antibiotics with outer and cytoplasmic membranes of *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy. 2000;**44**(12):3317-3321

[68] Lambert P. Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria. Journal of Applied Microbiology. 2002;**92**:46S-54S

[69] Stover C, Pham X, Erwin A, Mizoguchi S, Warren P, Hickey M, et al. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature. 2000;**406**(6799):959

[70] Dreier J, Ruggerone P. Interaction of antibacterial compounds with RND efflux pumps in *Pseudomonas aeruginosa*. Frontiers in Microbiology. 2015;**6**:660

[71] Morita Y, Nakashima K-I, Nishino K, Kotani K, Tomida J, Inoue M, et al. Berberine is a novel type efflux inhibitor which attenuates the MexXY-mediated

aminoglycoside resistance in *Pseudomonas aeruginosa*. Frontiers in Microbiology. 2016;**7**:1223

[72] Villegas MV, Lolans K, Correa A, Kattan JN, Lopez JA, Quinn JP, et al. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing β -lactamase. Antimicrobial Agents and Chemotherapy. 2007;**51**(4):1553-1555

[73] Jeannot K, Sobel ML, El Garch F, Poole K, Plésiat P. Induction of the MexXY efflux pump in *Pseudomonas aeruginosa* is dependent on drug-ribosome interaction. Journal of Bacteriology. 2005;**187**(15):5341-5346

[74] Chuanchuen R, Gaynor JB, Karkhoff-Schweizer R, Schweizer HP. Molecular characterization of MexL, the transcriptional repressor of the mexJK multidrug efflux operon in *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy. 2005;**49**(5):1844-1851

[75] Livermore DM, Winstanley TG, Shannon KP. Interpretative reading: Recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. Journal of Antimicrobial Chemotherapy. 2001;**48**(suppl_1):87-102

[76] Saiman L, Marshall BC, Mayer-Hamblett N, Burns JL, Quittner AL, Cibene DA, et al. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: A randomized controlled trial. Journal of the American Medical Association. 2003;**290**(13):1749-1756

[77] Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: Our worst nightmare? Clinical Infectious Diseases. 2002;**34**(5):634-640

[78] Zavascki AP, Carvalhaes CG, Picao RC, Gales AC. Multidrug-resistant *Pseudomonas aeruginosa* and

Acinetobacter baumannii: Resistance mechanisms and implications for therapy. Expert Review of Anti-Infective Therapy. 2010;8(1):71-93

[79] Poole K, Gilmour C, Farha MA, Parkins MD, Klinoski R, Brown ED. Meropenem potentiation of aminoglycoside activity against *Pseudomonas aeruginosa*: Involvement of the MexXY-OprM multidrug efflux system. Journal of Antimicrobial Chemotherapy. 2018;73(5):1247-1255

[80] Poole K. *Pseudomonas aeruginosa*: Resistance to the max. Frontiers in Microbiology. 2011;2:65

[81] Ochs MM, Bains M, Hancock RE. Role of putative loops 2 and 3 in imipenem passage through the specific porin OprD of *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy. 2000;44(7):1983-1985

[82] Lee J-Y, Ko KS. OprD mutations and inactivation, expression of efflux pumps and AmpC, and metallo- β -lactamases in carbapenem-resistant *Pseudomonas aeruginosa* isolates from South Korea. International Journal of Antimicrobial Agents. 2012;40(2):168-172

[83] Hancock RE, Speert DP. Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and impact on treatment. Drug Resistance Updates. 2000;3(4):247-255

[84] Fernández L, Hancock RE. Adaptive and mutational resistance: Role of porins and efflux pumps in drug resistance. Clinical Microbiology Reviews. 2012;25(4):661-681

[85] Breidenstein EB, de la Fuente-Núñez C, Hancock RE. *Pseudomonas aeruginosa*: All roads lead to resistance. Trends in Microbiology. 2011;19(8):419-426

[86] Fernández L, McPhee JB, Tamber S, Brazas MD, Lewenza S,

Hancock RE. Antibiotic resistance due to reduced uptake. In: Antimicrobial Drug Resistance. Cham: Springer; 2017. pp. 115-130

[87] Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. The Lancet. 2001;358(9276):135-138

[88] Mah T-F, Pitts B, Pellock B, Walker GC, Stewart PS, O'toole GA. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. Nature. 2003;426(6964):306

[89] Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: From the natural environment to infectious diseases. Nature Reviews. Microbiology. 2004;2(2):95

[90] Spoering AL, Lewis K. Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. Journal of Bacteriology. 2001;183(23):6746-6751

[91] Ma L, Conover M, Lu H, Parsek MR, Bayles K, Wozniak DJ. Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix. PLoS Pathogens. 2009;5(3):e1000354

[92] Moreau-Marquis S, Stanton BA, O'Toole GA. *Pseudomonas aeruginosa* biofilm formation in the cystic fibrosis airway. Pulmonary Pharmacology & Therapeutics. 2008;21(4):595-599

[93] Murray TS, Egan M, Kazmierczak BI. *Pseudomonas aeruginosa* chronic colonization in cystic fibrosis patients. Current Opinion in Pediatrics. 2007;19(1):83-88

[94] Webb JS, Thompson LS, James S, Charlton T, Tolker-Nielsen T, Koch B, et al. Cell death in *Pseudomonas aeruginosa* biofilm development. Journal of Bacteriology. 2003;185(15):4585-4592

- [95] Banin E, Vasil ML, Greenberg EP. Iron and *Pseudomonas aeruginosa* biofilm formation. Proceedings of the National Academy of Sciences. 2005;**102**(31):11076-11081
- [96] Ryder C, Byrd M, Wozniak DJ. Role of polysaccharides in *Pseudomonas aeruginosa* biofilm development. Current Opinion in Microbiology. 2007;**10**(6):644-648
- [97] Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, et al. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. The EMBO Journal. 2003;**22**(15):3803-3815
- [98] Diggle SP, Winzer K, Chhabra SR, Worrall KE, Cámara M, Williams P. The *Pseudomonas aeruginosa* quinolone signal molecule overcomes the cell density-dependency of the quorum sensing hierarchy, regulates rhl-dependent genes at the onset of stationary phase and can be produced in the absence of LasR. Molecular Microbiology. 2003;**50**(1):29-43
- [99] Dietrich LE, Price-Whelan A, Petersen A, Whiteley M, Newman DK. The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *Pseudomonas aeruginosa*. Molecular Microbiology. 2006;**61**(5):1308-1321
- [100] Parsek MR, Greenberg EP. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: A signaling mechanism involved in associations with higher organisms. Proceedings of the National Academy of Sciences. 2000;**97**(16):8789-8793
- [101] Rumbaugh KP, Griswold JA, Hamood AN. The role of quorum sensing in the in vivo virulence of *Pseudomonas aeruginosa*. Microbes and Infection. 2000;**2**(14):1721-1731
- [102] Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg E. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature. 2000;**407**(6805):762
- [103] Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science. 2000;**288**(5469):1251-1253
- [104] Hocquet D, Vogne C, El Garch F, Vejux A, Gotoh N, Lee A, et al. MexXY-OprM efflux pump is necessary for adaptive resistance of *Pseudomonas aeruginosa* to aminoglycosides. Antimicrobial Agents and Chemotherapy. 2003;**47**(4):1371-1375
- [105] Skiada A, Markogiannakis A, Plachouras D, Daikos GL. Adaptive resistance to cationic compounds in *Pseudomonas aeruginosa*. International Journal of Antimicrobial Agents. 2011;**37**(3):187-193
- [106] de la Fuente-Núñez C, Reffuveille F, Fernández L, Hancock RE. Bacterial biofilm development as a multicellular adaptation: Antibiotic resistance and new therapeutic strategies. Current Opinion in Microbiology. 2013;**16**(5):580-589
- [107] McPhee JB, Lewenza S, Hancock RE. Cationic antimicrobial peptides activate a two-component regulatory system, PmrA-PmrB, that regulates resistance to polymyxin B and cationic antimicrobial peptides in *Pseudomonas aeruginosa*. Molecular Microbiology. 2003;**50**(1):205-217
- [108] Fernández L, Gooderham WJ, Bains M, McPhee JB, Wiegand I, Hancock RE. Adaptive resistance to the “last hope” antibiotics polymyxin B and colistin in *Pseudomonas aeruginosa* is mediated by the novel two-component regulatory system

- ParR-ParS. Antimicrobial Agents and Chemotherapy. 2010;**54**(8):3372-3382
- [109] Sobel ML, McKay GA, Poole K. Contribution of the MexXY multidrug transporter to aminoglycoside resistance in *Pseudomonas aeruginosa* clinical isolates. Antimicrobial Agents and Chemotherapy. 2003;**47**(10):3202-3207
- [110] Smith EE, Buckley DG, Wu Z, Saenphimmachak C, Hoffman LR, D'Argenio DA, et al. Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. Proceedings of the National Academy of Sciences. 2006;**103**(22):8487-8492
- [111] Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. 2012;**18**(3):268-281
- [112] Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: Mechanisms and epidemiology. Clinical Microbiology and Infection. 2006;**12**(9):826-836
- [113] Levy SB, Marshall B. Antibacterial resistance worldwide: Causes, challenges and responses. Nature Medicine. 2004;**10**(12):S122-S129
- [114] Breidenstein EBM. Global Regulation of the Lon Protease of *Pseudomonas aeruginosa* and Its Influence on Ciprofloxacin Resistance and Virulence [thesis]. Vancouver, BC V6T 1Z4, Canada: University of British Columbia; 2012
- [115] Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrobial Agents and Chemotherapy. 2004;**48**(1):15-22
- [116] Gibb AP, Tribuddharat C, Moore RA, Louie TJ, Krulicki W, Livermore DM, et al. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new blaIMP allele, blaIMP-7. Antimicrobial Agents and Chemotherapy. 2002;**46**(1):255-258
- [117] Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: Mechanisms and epidemiology. International Journal of Antimicrobial Agents. 2015;**45**(6):568-585
- [118] Li X-Z, Nikaido H. Efflux-mediated drug resistance in bacteria. Drugs. 2004;**64**(2):159-204
- [119] Li X-Z, Zhang L, Poole K. Interplay between the MexA-MexB-OprM multidrug efflux system and the outer membrane barrier in the multiple antibiotic resistance of *Pseudomonas aeruginosa*. Journal of Antimicrobial Chemotherapy. 2000;**45**(4):433-436
- [120] Masuda N, Sakagawa E, Ohya S, Gotoh N, Tsujimoto H, Nishino T. Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM efflux pumps in *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy. 2000;**44**(12):3322-3327
- [121] Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: No ESKAPE! An update from the Infectious Diseases Society of America. Clinical Infectious Diseases. 2009;**48**(1):1-12
- [122] Vincent J-L, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. Journal of the American Medical Association. 2009;**302**(21):2323-2329

- [123] Xiao Y-H, Giske CG, Wei Z-Q, Shen P, Heddini A, Li L-J. Epidemiology and characteristics of antimicrobial resistance in China. *Drug Resistance Updates*. 2011;**14**(4-5):236-250
- [124] Crespo M, Woodford N, Sinclair A, Kaufmann M, Turton J, Glover J, et al. Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing VIM-8, a novel metallo- β -lactamase, in a tertiary care center in Cali, Colombia. *Journal of Clinical Microbiology*. 2004;**42**(11):5094-5101
- [125] Doring G, Conway S, Heijerman H, Hodson M, Hoiby N, Smyth A, et al. Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: A European consensus. *European Respiratory Journal*. 2000;**16**(4):749-767
- [126] Sekiguchi J-I, Asagi T, Miyoshi-Akiyama T, Kasai A, Mizuguchi Y, Araake M, et al. Outbreaks of multidrug-resistant *Pseudomonas aeruginosa* in community hospitals in Japan. *Journal of Clinical Microbiology*. 2007;**45**(3):979-989
- [127] Hughes AJ, Ariffin N, Huat TL, Molok HA, Hashim S, Sarijo J, et al. Prevalence of nosocomial infection and antibiotic use at a university medical center in Malaysia. *Infection Control and Hospital Epidemiology*. 2005;**26**(1):100-104
- [128] Rosenthal VD, Bijie H, Maki DG, Mehta Y, Apisarnthanarak A, Medeiros EA, et al. International Nosocomial Infection Control Consortium (INICC) report, data summary of 36 countries, for 2004-2009. *American Journal of Infection Control*. 2012;**40**(5):396-407
- [129] Buehrle DJ, Shields RK, Clarke LG, Potoski BA, Clancy CJ, Nguyen MH. Carbapenem-resistant *Pseudomonas aeruginosa* bacteremia: Risk factors for mortality and microbiologic treatment failure. *Antimicrobial Agents and Chemotherapy*. 2017;**61**(1):e01243-e01216
- [130] Trautmann M, Lepper PM, Haller M. Ecology of *Pseudomonas aeruginosa* in the intensive care unit and the evolving role of water outlets as a reservoir of the organism. *American Journal of Infection Control*. 2005;**33**(5):S41-S49
- [131] Hauser AR, Cobb E, Bodí M, Mariscal D, Vallés J, Engel JN, et al. Type III protein secretion is associated with poor clinical outcomes in patients with ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*. *Critical Care Medicine*. 2002;**30**(3):521-528
- [132] Rahal JJ, Urban C, Segal-Maurer S. Nosocomial antibiotic resistance in multiple Gram-negative species: Experience at one hospital with squeezing the resistance balloon at multiple sites. *Clinical Infectious Diseases*. 2002;**34**(4):499-503
- [133] Reis AO, Cordeiro JC, Machado AM, Sader HS. In vitro antimicrobial activity of linezolid tested against vancomycin-resistant enterococci isolated in Brazilian hospitals. *Brazilian Journal of Infectious Diseases*. 2001;**5**(5):243-251
- [134] Zhanel GG, Mayer M, Laing N, Adam HJ. Mutant prevention concentrations of levofloxacin alone and in combination with azithromycin, ceftazidime, colistin (Polymyxin E), meropenem, piperacillin-tazobactam, and tobramycin against *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*. 2006;**50**(6):2228-2230
- [135] Nichols DP, Happoldt CL, Bratcher PE, Caceres SM, Chmiel JF, Malcolm KC, et al. Impact of azithromycin on the clinical and antimicrobial effectiveness of tobramycin in the treatment of cystic

fibrosis. *Journal of Cystic Fibrosis*. 2017;**16**(3):358-366

[136] Barraud N, Hassett DJ, Hwang S-H, Rice SA, Kjelleberg S, Webb JS. Involvement of nitric oxide in biofilm dispersal of *Pseudomonas aeruginosa*. *Journal of Bacteriology*. 2006;**188**(21):7344-7353

[137] Matsuo Y, Eda S, Gotoh N, Yoshihara E, Nakae T. MexZ-mediated regulation of mexXY multidrug efflux pump expression in *Pseudomonas aeruginosa* by binding on the mexZ-mexX intergenic DNA. *FEMS Microbiology Letters*. 2004;**238**(1):23-28

[138] Schuster M, Lostroh CP, Ogi T, Greenberg EP. Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: A transcriptome analysis. *Journal of Bacteriology*. 2003;**185**(7):2066-2079

[139] Nuri R, Shprung T, Shai Y. Defensive remodeling: How bacterial surface properties and biofilm formation promote resistance to antimicrobial peptides. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. 2015;**1848**(11):3089-3100

[140] Schurek KN, Sampaio JL, Kiffer CR, Sinto S, Mendes CM, Hancock RE. Involvement of pmrAB and phoPQ in polymyxin B adaptation and inducible resistance in non-cystic fibrosis clinical isolates of *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*. 2009;**53**(10):4345-4351

[141] Priyadarsini S, Mohanty S, Mukherjee S, Basu S, Mishra M. Graphene and graphene oxide as nanomaterials for medicine and biology application. *Journal of Nanostructure in Chemistry*. 2018;**8**(2):123-137

[142] Taylor PK, Yeung AT, Hancock RE. Antibiotic resistance

in *Pseudomonas aeruginosa* biofilms: Towards the development of novel anti-biofilm therapies. *Journal of Biotechnology*. 2014;**191**:121-130

[143] Jansen KU, Anderson AS. The role of vaccines in fighting antimicrobial resistance (AMR). *Human Vaccines & Immunotherapeutics*. 2018;**14**(9):2142-2149

[144] Merakou C, Schaefer MM, Priebe GP. Progress toward the elusive *Pseudomonas aeruginosa* vaccine. *Surgical Infections*. 2018;**19**(8):757-768

[145] Pang Z, Raudonis R, Glick BR, Lin T-J, Cheng Z. Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and alternative therapeutic strategies. *Biotechnology Advances*. 2019;**37**(1):177-192