Chapter 7
Resistance and Response to Anti-Pseudomonas Agents and Biocides

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Abstract *Pseudomonas aeruginosa* possesses an innate resistance to various antimicrobial agents, owing much to its low membrane permeability and multidrug efflux pumps. Although anti-pseudomonas agents are effective against this organism, their repeated use has been linked to the development of drug resistance with the emergence of multidrug resistant *Pseudomonas aeruginosa* strains in clinical settings of special concern. Inappropriate use of disinfectants can be also causes of *P. aeruginosa* infection.

Multidrug resistance in *P. aeruginosa* is complex, and attributable to both chromosomal mutations and the acquisition of extraneous resistance genes. Recent genome-wide analyses of *P. aeruginosa* have uncovered novel intrinsic resistance mechanisms and global responses to antimicrobials. Among these strategies, two in particular deserve special mention: two-component systems, and intracellular proteases.

Introduction

Pseudomonas aeruginosa possesses an intrinsic resistance to many antimicrobials, owing to the low permeability of its outer membrane barrier and the presence of multidrug efflux transporters (Poole 2011). Although broad-spectrum β-lactams (e.g., imipenem), fluoroquinolones (e.g., ciprofloxacin), and anti-pseudomonas aminoglycosides (e.g., amikacin) are often available for treatment, *P. aeruginosa* readily acquires resistance to these specific agents via chromosomal mutations and lateral gene transfer. As only a limited number of new antimicrobial agents are in development, the emergence and spread of multidrug resistant *P. aeruginosa* infections, against which very few agents are currently effective, is of great concern. Here we discuss how *P. aeruginosa* responds to anti-pseudomonas agents to survive as a pathogen.

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Carbapenems and Other Anti-Pseudomonas β-Lactams

β-lactams bind to cell wall transpeptidases (penicillin binding proteins (PBPs)) blocking an important step in peptideglycan biosynthesis (Poole 2004). Among anti-pseudomonas β-lactams, carbapenems (e.g., imipenem and meropenem) are an important class of anti-pseudomonal β-lactams owing to their stability when exposed to most β-lactamases. Under clinical conditions, the acquisition of extraneous carbapenases, especially metallo-β-lactamases (MBLs) is of greatest concern, as it is the major cause of high-level resistance to almost all β-lactams, including carbapenems. This resistance is then frequently co-transferred with genes encoding aminoglycoside-modifying enzymes (or rarely 16S rRNA methylases) (Poole 2011). The most common mechanism through which carbapenem resistance is acquired is loss or alteration of the outer membrane OprD porin protein (e.g., Shu et al. 2012; Khuntayaporn et al. 2013; Fournier et al. 2013), the major portal for entry for carbapenems (Trias and Nikaido 1990). Other additional mechanisms such as resistance, nodulation, and cell division (RND)-type multidrug efflux pumps (such as MexAB/MexXY-OprM and/or AmpC β-lactamase) further strengthen their resistance (e.g., Riera et al. 2011).

Causes responsible for the loss of porin OprD in *P. aeruginosa* are a lack or alteration of OprD production through disruption of *oprD*'s coding or upstream regions and downregulated OprD production through mutations of the two-component ParRS system, the two-component CzcRS system, and the probable oxidoreductase MexS with inverse coregulation of active RND-type efflux systems (Fournier et al. 2013). Notably, some amino acid substitutions of the ParRS system cause not only downregulation of *oprD*, but also upregulation of the RND-efflux *mexXY* pump gene and lipopolysaccharide modification *arnBCADTEF-ugd* operon, interconnecting resistance to polymyxins, aminoglycosides, fluoroquinolones, and some β-lactams (e.g., carbapenems and cefepime) (Muller et al. 2011). Additionally, some amino acid substitutions of the MexS causes downregulation of *oprD* and upregulation of RND-efflux *mexEF-oprN* pump genes, both of which depend on the activator gene *mexT,* yielding increased resistance to carbapenems and fluoroquinolones (Sobel et al. 2005). Sub-inhibitory concentrations of imipenem were shown to strongly induce *ampC* gene expression both in planktonic cells and biofilm, although imipenem is not itself a substrate of the AmpC enzyme (Bagge et al. 2004). In addition, *P. aeruginosa* biofilms exposed to imipenem induced genes coding for alginate biosynthesis, causing thicker and more robust biofilms (Bagge et al. 2004).

In addition to carbapenems, penicillins (e.g., ticarcillin, piperacillin), cephalosporin (e.g., ceftazidime, cefepime) and monobactams (e.g., aztreonam) are commonly used to treat pseudomonal infection. Mutational de-repression of *ampC* is the most common mechanism of β-lactam resistance (Poole 2011). High-level clinical β-lactams (except to carbapenems) resistance was shown to be driven by inactivation of *dacB*-encoded nonessential PBP4, triggering overproduction of the chromosomal β-lactamase AmpC and the specific activation of CreBC (BlrAB) two-component regulator (Moya et al. 2009). Among the RND multidrug efflux pumps,

MexAB-OprM accommodates the broadest range of β-lactams (e.g., ticarcillin, piperacillin, ceftazidime, and aztreonam) and is most frequently linked to β-lactam resistance, although MexXY-OprM has been linked to some β-lactams (e.g., cefepime) as well (Hocquet et al. 2006; Poole 2011). Ceftazidime, a PBP3 inhibitor, which does not induce *ampC* gene expression, but is rather a substrate of AmpC, impacts the transcription of a large number of genes in *P. aeruginosa,* including those of SOS response repressor LexA-like proteins (Blázquez et al. 2006). Noticeably, this antimicrobial agent induces the error-prone DNA polymerase DinB, causing induced mutagenesis and decreasing ciprofloxacin toxicity (Blázquez et al. 2006). The acquisition of extraneous β-lactamases including ESBL frequently co-transferring with genes encoding aminoglycoside-modifying enzymes also deserves attention (Poole 2011).

The mutants' profiles of susceptibility for three β-lactams, namely ceftazidime, imipenem, and meropenem, suggested that a wide array of elements, but distinct mechanisms of action and resistance contributes to β-lactam resistance despite these compounds belonging to the same structural family (Alvarez-Ortega et al. 2010). Only three mutants demonstrated reduced (PA0908) or increased (*glnK* and *ftsK*) susceptibility to all three β-lactams (Alvarez-Ortega et al. 2010). The mechanisms involved vary, as *glnK* encodes a nitrogen regulatory protein PII-2 and *ftsK* encodes a protein involved in cell division and stress response (Alvarez-Ortega et al. 2010).

Fluoroquinolones

Fluoroquinolones, particularly ciprofloxacin, are commonly used in the treatment of *P. aeruginosa* infections (Poole 2011). This class of agents interacts with complexes composed of DNA and either of the two target enzymes, DNA gyrase or topoisomerase IV (Hooper 1998). Resistance to these agents, particularly high-level resistance, is predominantly mediated by mutations in these flouroquinolones-targeted enzymes, though efflux is a significant contributing factor, often in combination with target site mutations (Poole 2011). The four RND-type multidrug efflux pumps (MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM) are well recognized as significant determinants of fluoroquinolone resistance (Poole 2011) although fluoroquinolone-resistant mutants which overproduce other RND multidrug efflux pumps might exist. Some, such as MexGH-OpmD and MexVW can be isolated from mutants lacking the four major RND multidrug efflux pumps (Sekiya et al. 2003; Li et al. 2003) and other efflux pump-bolstering fluoroquinolone resistance may be coded for in the genome (e.g., NorM ortholog of *P. aeruginosa* (Morita et al. 1998)).

The *mexAB-oprM* operon is constitutively expressed in wild-type cells under usual laboratory conditions, where it contributes to *P. aeruginosa's* intrinsic resistance to quinolones and other many antimicrobial agents (e.g., carbenicillin, aztreonam, chloramphenicol, and tetracycline) (Poole 2013; Morita et al. 2001). Highly

expressed MexAB-OprM quinolone-resistant mutants are classified as *nalB*, *nalC*, or *nalD*. *NalB* mutants carry lesions in the *mexR* gene encoding a local repressor of the *mexAB-oprM* operon (Srikumar et al. 2000). *NalC* mutants carry a mutation in a local *nalC* repressor gene of an adjacent two-gene operon, PA3720-PA3721 (Cao et al. 2004). PA3721 was named *armR* after its function as an anti-repressor of MexR, where AmrR binds to MexR and negatively impacts *mexAB-oprM* expression (Daigle et al. 2007). While MexR binds to the first (efflux-operon distant) promoter (Morita et al. 2006a), yet a third gene, *nalD* also encodes a repressor, but instead binds to the second (efflux-operon proximal) promoter upstream of *mexAB-oprM*. The *mexCD-oprJ* operon and the *mexEF-oprN* operon are almost unnoticeable in wild type cells and are overexpressed in the quinolone-resistant mutants known as *nfxB* and *nfxC,* respectively (Poole 2013; Kohler et al. 1997). *NfxB* mutants, which carry lesions in their local repressor gene, *nfxB,* of the *mexCD-oprJ* operon, showed increased resistance to antimicrobial agents including fluoroquinolone and zwitterionic cephems, and decreased resistance to both various β-lactams (ticarcillin, cefepime, ceftazidime, and aztreonam) and aminoglycosides (Poole 2013 Jeannot et al. 2008). Notably, MexCD-OprJ-overproducing mutants are rarely seen under clinical conditions (Jeannot et al. 2008). *NfxC* mutants which carry lesions in the local activator *mexT* gene of the *mexEF-oprN* operon, or the probable oxidoreductase *mexS* gene showed increased resistance to antimicrobial agents including fluoroquinolone and carbapenems, and decreased resistance to various β-lactams (ticarcillin, cefepime, ceftazidime, and aztreonam) and aminoglycosides (Sobel et al. 2005; Llanes et al. 2011). The molecular mechanisms of *mexEF-oprN* overexpression in *nfxC* mutants are described above. Although the expression of *mexXY* in wild type cells is repressed by *mexZ,* a cognate regulator gene, overexpression of *mexXY* increased the level of fluoroquinolones resistance in *P. aeruginosa* cells (Aires et al. 1999; Morita et al. 2001). The incidence of MexXY-OprM overproducers among clinical isolates has been linked to the use of various antibiotics, including ciprofloxacin, cefepime and amikacin, but not imipenem (Hocquet et al. 2008).

Transcriptional responses of *P. aeruginosa* to sub-inhibitory and inhibitory ciprofloxacin exposure demonstrated the expression of hundreds of promoted or repressed genes (Brazas and Hancock 2005; Cirz et al. 2006; Brazas et al. 2007). Surprisingly, genes for bacteriophage-like pyocins were upregulated and mediated fluoroquinolone susceptibility (Brazas and Hancock 2005). At least one-third of upregulated genes occur in regulons that are likely controlled by LexA-like SOS response repressor proteins in response to inhibitory concentrations of ciprofloxacin, while downregulated genes appear to involve virtually every facet of cellular metabolism (Cirz et al. 2006; Brazas et al. 2007). The overall pattern of expression in the DNA replication enzymes suggests a shift from canonical DNA replication enzymes to inducible polymerases in response to inhibitory ciprofloxacin concentrations (Cirz et al. 2006). These inhibitory concentrations of ciprofloxacin create selection pressure in favor of mutants with increased *ampC* expression (Wolter et al. 2007), while sub-inhibitory levels of ciprofloxacin or ofloxacin enhance the mutation frequency for carbapenem (esp., meropenem)-selected carbapenem resistance (Tanimoto et al. 2008).

Several mutants with increased or decreased ciprofloxacin susceptibilities were identified through random transposon insertion libraries, highlighting complex ciprofloxacin resistomes (Brazas et al. 2007; Breidenstein et al. 2008). Among the ciprofloxacin mutants with increased susceptibility (revealing genes involved in intrinsic resistance) were an exceptionally high number of mutants with mutations involving DNA replication and repair (Brazas et al. 2007; Breidenstein et al. 2008). Among the most unusual were mutants with alterations in *ftsK* encoding DNA segregation ATPase (Breidenstein et al. 2008) and *lon* encoding ATP-dependent intracellular protease, respectively (Brazas et al. 2007; Breidenstein et al. 2008). The *ftsK* mutants also showed increased susceptibility to the β-lactams described above (Alvarez-Ortega et al. 2010). Lon modulates SOS response and consequently ciprofloxacin susceptibility (Breidenstein et al. 2012).

Aminoglycosides

Aminoglycosides bind to the 30S ribosomal subunit and interfere with protein synthesis, causing mistranslation and ultimately cell death without lysis (Davis 1987). Anti-pseudomonas aminoglycosides (e.g., amikacin, gentamicin, and tobramycin) can therefore be used in the treatment of *Pseudomonas aeruginosa* infections (Poole 2005). Aminoglycoside uptake and subsequent action within bacterial cells is a complex process that involves lipopolysaccharide binding and outer membrane permeation, cytoplasmic membrane (CM) traversal driven by membrane potential, and ribosome disruption, leading to the production of membrane-damaging mistranslated polypeptides (Davis 1987; Krahn et al. 2012). The antagonism of aminoglycosides by divalent cations Mg^{2+} and Ca^{2+} is well documented in *P. aeruginosa*, which requires a MexXY multidrug efflux system (Mao et al. 2001; Morita et al. 2012b).

Aminoglycoside resistance typically results from the acquisition of aminoglycosides-modifying enzymes (AMEs) the genes for which are typically found on MBL-containing integrons in multidrug resistant *P. aeruginosa* (Poole 2011). Upregulation of the MexXY multidrug efflux system, in addition to AME acquisition, leads to *P. aeruginosa* developing high levels of aminoglycoside resistance (Morita et al. 2012b). Extremely high resistance to all anti-pseudomonas aminoglycosides is also promoted by 16S rRNA methylases (RMTs), recently discovered in *P. aeruginosa* but rarely observed (Poole 2011). This is not, however, the case for cystic fibrosis (CF) patients of isolates, where the mechanism are almost unknown but the most commonly observed aminoglycoside adaptive resistance phenotype of *P. aeruginosa* is impermeability phenotype and up-regulation of the MexXY multidrug efflux pump is predominant (Poole 2011). In wild type *P. aeruginosa* cells, the MexXY efflux system is inducible with sub-inhibitory concentrations of aminoglycoside- and ribosome-targeting antimicrobials (e.g., chloramphenicol and tetracycline), which are involved in the gene product PA5471 (recently dubbed ArmZ for anti-repressor MexZ (Hay et al. 2013)) (Morita et al. 2006b). The PA5471 system is also inducible

through interference, with translation of the leader peptide PA5471.1 (Morita et al. 2009). Overall the MexXY system has been increasingly recognized as the favored determinant of aminoglycoside resistance (Morita et al. 2012a).

P. aeruginosa possesses and exploits a great number of genes (at least 135) the inactivation of which contributes to low-level aminoglycoside resistances through genome-wide analysis (Schurek et al. 2008). These genes were involved predominantly in energy metabolism, which is reasonable when considering that aminoglycoside uptake by bacteria is energy-dependent (Schurek et al. 2008, p. 5). In previous studies, most of these mutant strains did not exhibit growth defects under the conditions tested, although some exhibited a small-colony phenotype and/or growth defects under anaerobic conditions (Schurek et al. 2008), Several additional genes involved in LPS biosynthesis which contribute to outer membrane permeability were also identified (Schurek et al. 2008). Schurek's results are consistent with previous reports on both impermeability and adaptive aminoglycoside resistance (Poole 2005). Furthermore, the deletion of such genes compromised the high level pan-aminoglycoside resistance of clinical isolates, emphasizing their importance to acquired resistance (Krahn et al. 2002).

Genome-scale identification of intrinsic aminoglycoside resistance in *P. aeruginosa* has also been performed (Lee et al. 2009; Struble and Gill 2009; Gallagher et al. 2011; Krahn et al. 2002). Although there are many newly identified intrinsic aminoglycoside determinants, the most noteworthy include an AmgRS two-component system, potassium, phosphate transporters (Trk and Pts systems), lipid biosynthesis or metabolism (LptA and FaoA), and membrane protein folding (PpiD) (Lee et al. 2009; Struble and Gill 2009; Gallagher et al. 2011; Krahn et al. 2012). The AmgRS two-component system was shown to control an adaptive response to membrane stress, which can be caused by aminoglycoside-induced translational misreading, in order to enhance aminoglycoside susceptibility in planktonic cells and biofilm (Lee et al. 2009; Krahn et al. 2012). Interestingly, mutational activation of AmgRS was recently found in pan-aminoglycoside resistant *P. aeruginosa* clinical isolates (Lau et al. 2013). AmgRS strongly regulated the expression of several genes involved in proteolysis (*htpX, nlpD,* and *yccA*), membrane transport (*yegH, ygiT,* and *sugE*) and other functions (*yebE*, PA5528, *yceJ*), but not the MexXY multidrug efflux pump (Lee et al. 2009). The combined effects of three genes (*yccA, htpX,* and PA5528) largely accounts for AmgRS-controlled resistance (Hinz et al. 2011). The first, *yccA*, encodes a protein whose homologue modulates the activity of membrane protease FtsH; the second, *htpX*, encodes a membrane protease itself; and the third, PA5528, encodes a membrane protein of unknown function, indicating that proteolysis plays a central part in aminoglycoside resistance (Hinz et al. 2011). The membrane protease FtsH was shown to be particularly important to *P. aeruginosa*'s aminoglycoside resistance (Hinz et al. 2011). The network of proteases provides robust protection from aminoglycosides and other substances through the elimination of membrane-disruptive mistranslation products (Hinz et al. 2011).

Transcriptomic analyses confirm that aminoglycosides impact the expression of a myriad of genes (Kindrachuk et al. 2011). While prolonged exposure to subinhibitory concentrations of tobramycin caused increased levels of expression pre-

dominantly of the *mexXY* efflux pump genes, the greatest increases in gene expression levels in response to lethal concentrations of tobramycin involved a number of *P. aeruginosa*'s heat shock genes (e.g., *htpG*, *ibpA*, *groES,* and *asrA*) (Kindrachuk et al. 2011). Under these conditions, the likely intracellular ATP-dependent AsrA protease is noteworthy because of a modest positive impact on aminoglycoside resistance (Kindrachuk et al. 2011). The Lon protease is also inducible by aminoglycoside (Marr et al. 2007).

Sub-inhibitory concentrations of aminoglycosides, especially tobramycin, induce biofilm formation in *P. aeruginosa* (Hoffman et al. 2005). Aminoglycoside response regulator (Arr) is predicted to encode an inner membrane phosphodiesterase whose substrate is cyclic di-guanosine monophosphate (c-di-GMP). A bacterial second messenger that regulates cell surface adhesiveness is essential for this induction and contributes to biofilm-specific aminoglycoside resistance (Hoffman et al. 2005). *ndvB* and PA1874-1877 genes involved in biofilm-specific resistance to aminogylycosides and other compounds (e.g., fluoroquinolone) have also been reported (Mah et al. 2003; Zhang and Mah 2008). The *ndvB* gene is involved in the production of highly glycerol-phosphorylated β-(1→3)-glucans, which bind to aminoglycosides (Sadovskaya et al. 2009) and affects the expression of multiple genes in biofilms. Ethanol oxidation genes are also linked to biofilm-specific antibiotic resistance (Beaudoin et al. 2012)

Polymyxins

Owing to the increased prevalence of multidrug-resistant *P. aeruginosa,* polymyxin B and colistin (also called polymyxin E), belonging to a family of antimicrobial cyclic oligopeptides, have returned to favor as a last resort treatment option, although these agents have strong side effects (e.g., nephrotoxicity) with high incidence (Poole 2011). The mechanism of polymyxins involves an initial stage of interaction with the lipid A of lipopolysaccharides (LPS), leading to self-promoted uptake across the membrane, followed by cell death (Zhang et al. 2000; Fernandez et al. 2013). The most common resistance mechanism to polymyxin has been shown to arise from substitution of LPS lipid A with 4-amino-L-arabinose *in vitro* and in cystic fibrosis isolates, while other unknown mechanisms remain under investigation (Poole 2011; Miller et al. 2011; Moskowitz et al. 2012). This modification is carried out by the products of the *arnBCADTEF-ugd* operon, otherwise known as *pmrHFIJKLM-ugd* (McPhee et al. 2003; Yan et al. 2007). Expression of the *arn* operon is activated by a series of two-component systems (involving at least PhoPQ, PmrAB, ParRS, and CprRS) after recognizing environmental signals including low Mg^{2+} (PhoPQ and PmrAB) or cationic peptides (ParRS and CprRS) (Macfarlane et al. 2000; McPhee et al. 2003; Fernandez et al. 2010, 2012), or when mutations in *phoQ*, *pmrB*, and *parR* promote low to moderate polymyxin resistance (Miller et al. 2011; Moskowitz et al. 2012; Muller et al. 2011). The set of genes directly or indirectly responding to mutated ParRS included the *pmrAB* genes (Muller et al. 2011),

consistent with previous data that polymixin induced *pmrAB* gene expression to a weaker extent than did other antimicrobial catioinic peptides or low Mg^{2+} (McPhee et al. 2003). Polymixin B resistance in *P. aeruginosa* is fairly limited and includes LPS-related genes (*galU*, *lptC*, *wapR,* and *ssg*) (Fernandez et al. 2013). Recently, polymyxin resistance in *P. aeruginosa phoQ* mutants was shown to be dependent on additional two-component systems: ColRS and CprRS. The addition of 4-amino-L-arabinose to lipid A is not the only mechanism to acquire this resistance, (Gutu et al. 2013), although it results in a less virulent but more inflammatory phenotype (Gellatly et al. 2012).

Approximately 0.5% of genes showed significantly altered expression in the sub-inhibitory concentration of colistin (Cummins et al. 2009), which is no less dramatic than the other anti-pseudomonas agents (e.g., ceftazidime, ciprofloxacin, and tobramycin) described above. The most noticeable alternation was upregulation of Pseudomonas quinolone signal (PQS) biosynthetic genes such as the *pqsABCDE* operon, the phenazine biosynthetic operon, or the *arn* operon (Cummins et al. 2009).

Development of colistin tolerance was observed in spatially distinct sub-populations of metabolically active *P. aeruginosa* cells on a biofilm, unlike those exhibiting low metabolic activity and dependence on the *arn* and efflux pump operons (*mexAB-oprM*, *mexCD-oprJ*, and *muxABC-opmB*) (Pamp et al. 2008; Chiang et al. 2012). MuxABC-OpmB, originally characterized as a multidrug efflux pump only in mutants lacking the major RND multidrug efflux pumps (Mima et al. 2009), was shown to contribute to intrinsic carbenicillin resistance and twitching motility of *P. aeruginosa* cells (Yang et al. 2011).

Macrolides

Macrolides such as erythromycin and azithromycin are widely used antibiotics which block translation by binding to the 50S ribosomal subunit. While *P. aeruginosa* cells are intrinsically very resistant to macrolides in standard broths such as Mueller-Hinton, low-dosage macrolides such as azithromycin are effective treatments in patients with chronic lung infections (Kudoh et al. 1998; Jaffe et al. 1998). Macrolides (e.g., azithromycin, erythromycin, clarithromycin) even far below their MICs (e.g., 2 μg/ml of azithromycin) exhibit exposure-dependent bactericidal effects to inhibit protein and autoinducer synthesis, leading to a reduction in virulence factor production (Tateda et al. 1996, 2001). Genome-wide approaches uncover the quorum-sensing antagonistic activities of azithromycin (i.e., inhibition of quorum sensing, reduction of virulence factor production and strong induction of type III secretion systems) (Nalca et al. 2006). This modulation causes downregulation of MexAB-OprM efflux pump expression in *P. aeruginosa* (Sugimura et al. 2008). Sub-MIC levels of azithromycin does not affect mRNA expression of quorum-sensing-related genes (*lasI*, *lasR*, *rhlI*, *rhlR*, *vft*, and *rsaL*), but lowers expression of most N-acylhomoserine lactone (AHL) synthesis enzymes, which act upstream

of *lasI* or *rhlI* (Kai et al. 2009). Azithromycin additionally inhibits expression of the small RNAs RsmY and RsmZ, dependent on the GacA/Rsm signal transduction pathway which positively controls *P. aeruginosa* quorum sensing (Perez-Martinez and Haas 2011).

Both effects of azithromycin on quorum-sensing dependent virulence factor production and cell death requires azithromycin to interact with ribosomes (Kohler et al. 2007). The stationary-phase killing of azithromycin is further enhanced by the production of rhamnolipids, which likely facilitate macrolide uptake (Kohler et al. 2007). The mode of action of azithromycin *in vivo* is also described through mutations in 23S rRNA conferring azithromycin resistance to cystic fibrosis patients chronically infected with *P. aeruginosa* (Marvig et al. 2012). The clinical efficacy of macrolides in treating pseudomonal infections can be partially explained by the increased susceptibility of *P. aeruginosa* to these compounds in eukaryotic cell culture media and biological fluids, due to decreased *oprM* expression of and increased outer-membrane permeability (Buyck et al. 2012).

Biocides

P. aeruginosa has also been reported to contaminate disinfectants (e.g., chlorhexidine, benzalkonium, and triclosan) in hospital or other such environments, thereby compromising their ability to reduce or eliminate bacterial contamination. Chlorhexidine and benzalkonium are a cationic biguanide and a nitrogen-based quaternary ammonium compound, respectively. They function by affecting the cell membrane, resulting in lysis and the loss of cytoplasmic material (Morita et al. 2014). The RND-type MexCD-OprJ multidrug efflux pump is inducible at sub-inhibitory concentrations of disinfectants such as benzalkonium chloride or chlorhexidine (Morita et al. 2003), dependent upon the AlgU stress response factor (Fraud et al. 2008). Global transcriptome response to chlorhexidine included upregulation of the *mexCD-oprD* and *oprH-phoPQ* operons and downregulation of membrane transport, oxidative phosphorylation electron transport and DNA repairs (Nde et al. 2009). The variant highly adapted to benzalkonium showed increased resistance to fluoroquinolones, owing to mutations in the quinolone resistance-determining region of *gyrA* and repressor genes (*mexR* and *nfxB*) of *mexAB-oprM* and *mexCD-oprJ*, respectively (Mc Cay et al. 2010). Development of chlorhexidine-tolerant sub-populations on the *P. aeruginosa* biofilm was also dependent on the *mexCD-oprJ* genes (Chiang et al. 2012).

Triclosan specifically inhibits fatty acid synthesis through inhibition of bacterial enoyl-acyl carrier protein reductase, although *P. aeruginosa* is intrinsically highly resistant to triclosan due to FabV (triclosan resistant enoyl-acyl carrier protein reductase) and active efflux (Zhu et al. 2010). This innate resistance stems from at least five RND efflux pumps including MexAB-OprM (Zhu et al. 2010; Mima et al. 2007). In *P. aeruginosa* mutant cells lacking the *mexAB-oprM* genes, sub-inhibitory concentrations of triclosan led to alterations in almost half the genome, with 28%

of genes being significantly upregulated and 16% being significantly downregulated (Chuanchuen and Schweizer 2012). Quorum sensing-regulating genes were among the most strongly downregulated, and surprisingly, iron homeostasis was completely blocked in triclosan-exposed cells, thus mimicking conditions with excess iron (Chuanchuen and Schweizer 2012).

Further Prospects

Our findings regarding mode of action, resistance mechanisms, and global responses to antimicrobial agents paves the ways to conquer *P. aeuginosa* infections. We are aware of potential molecular targets for novel genomic anti-pseudomonas agents, including essential gene products (Morita et al. 2010). However, we first need to address the problems of low membrane permeability and multidrug efflux pumps in *P. aeruginsoa* to develop these novel agents. While many labs, including our own, are currently screening, so far there are no efflux pump inhibitors available for clinical settings and screening novel antibacterial agents, including an efflux inhibitor, is currently in progress in many labs including ours.

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