Responses of Pseudomonas aeruginosa to antimicrobials

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INTRODUCTION
Pseudomonas aeruginosa, a motile, non-fermenting Gram-negative bacterium, is an opportunistic pathogen implicated in respiratory infections, urinary tract infections, gastrointestinal infections, keratitis, otitis media, and bacteremia in patients with compromised host defenses (e.g., cancer, burn, HIV, and cystic fibrosis (CF)). These infections often result in significant morbidity and mortality. In the 21st century, when the life expectancy of highly susceptible immunocompromised groups has been extended in most countries, P. aeruginosa plays an increasingly prominent role in hospital infections.

This organism is a ubiquitous and metabolically versatile microbe that flourishes in many environments. The bacterium grows under both aerobic and anaerobic conditions, and possesses numerous virulence factors that contribute to its pathogenesis (Schurr et al., 2012). Moreover P. aeruginosa possesses an intrinsically resistant to many antimicrobials because of the bacterium’s outer-membrane barrier, the presence of multidrug efflux pumps, and endogenous antimicrobial inactivation (Poole, 2011). Although anti-Pseudomonas agents (e.g., carbapenems) have been discovered and developed, P. aeruginosa readily acquires resistance to individual agents via chromosomal mutations and lateral gene transfer (Poole, 2011).

Pseudomonas aeruginosa possesses multifactorial mechanisms of responses and resistance to antimicrobials. While antimicrobials were originally developed and used to kill bacteria, recent work reveals that the biological functions of antibiotics are not limited to bactericidal (killing) or bacteriostatic (growth inhibition) effects (Linares et al., 2006; Aminov, 2013). The most likely function of antibiotics in natural ecosystems is in intercellular “signaling,” with specific consequences on the collective behavior of the bacterial population (Linares et al., 2006; Aminov, 2013).

Improved genetic tools and cutting-edge technologies (e.g., DNA microarrays) have revolutionized our understanding of microbial physiology (Wecke and Mascher, 2011). Here, we summarize and discuss how P. aeruginosa responds to various antimicrobials and survives against its competitors.

RESPONSES TO β-LACTAMS
β-lactams bind to cell wall transpeptidases (penicillin binding proteins (PBPs)), blocking an important step in peptidoglycan biosynthesis (Poole, 2004). Penicillins (e.g., ticarcillin, piperacillin),

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cephalosporins (e.g., ceftazidine, ceftepime), monobactams (e.g., aztreonam), and carbapenems (e.g., imipenem, meropenem, and doripenem) are commonly used to treat pseudomonal infections. *P. aeruginosa* is intrinsically resistant to most β-lactams due to the interplay of the inducible β-lactamase AmpC and the resistance nodulation cell division (RND) multidrug efflux systems (e.g., MexAB-OprM; Morita et al., 1999). Benzyl-penicillins (e.g., amoxicillin) and narrow-spectrum cephalosporins are labile to hydrolysis and are strong inducers of *ampC*, leading to antibiotic degradation, whereas ureidopenicillins (e.g., piperacillin) and extended-spectrum cephalosporins are labile but are weak inducers of *ampC* (Livermore, 1995). The MexAB-OprM operon is constitutively expressed in wild-type cells under usual laboratory conditions, where the operon contributes to *P. aeruginosa*'s intrinsic resistance to most β-lactams (except for imipenem) and many other antimicrobial agents, including quinolones, tetracycline, chloramphenicol, and macrolides (Morita et al., 2001). Blocking of dacit-encoded non-essential PBP4 determines a highly efficient and complex β-lactam resistance response, triggering the overproduction of AmpC and the specific activation of the CepAB (BlaB) two-component regulator (Moya et al., 2009).

Carbapenems are an important class of anti-pseudomonal β-lactams. Carbapenems are strong inducers that are marginally labile (imipenem) or effectively stable (meropenem; Livermore, 1995). Imipenem also has been shown to strongly induce *ampC* gene expression in biofilms (Bagge et al., 2004). In addition, *P. aeruginosa* biofilms exposed to imipenem exhibit elevated expression of genes coding for alginate biosynthesis, causing thicker and more robust biofilms (Bagge et al., 2004).

Ceftazidine, a PBP3 inhibitor, does not induce *ampC* gene expression, but is rather a substrate of AmpC. Ceftazidine impacts the transcription of a large number of genes in *P. aeruginosa*, including those encoding the SOS response repressor LexA-like proteins, causing induced mutagenesis and decreasing ciprofloxacin toxicity (Blazquez et al., 2006). In addition, this antimicrobial shows quorum sensing (QS) inhibitory activity, decreasing the production of a range of QS-regulated virulence factors, in contrast to piperacillin, another PBP3 inhibitor (Skinderoe et al., 2008). These results imply that the QS inhibitory activity of ceftazidine is likely not PBP3-dependent (Skinderoe et al., 2008).

**RESPONSES TO FLUOROQUINOLONES**

Fluoroquinolones, particularly ciprofloxacin, are commonly used for 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 and/or topoisomerase IV. Primary intrinsic resistance of the wild-type *P. aeruginosa* to fluoroquinolones is due to MexAB-OprM as well as to MexXY-OprM (Morita et al., 2001). The four RND-type multidrug efflux pumps (MexAB-OprM, MexCD-OprM, MexEF-OpsN, and MexXY-OprM) are well recognized as significant determinants of fluoroquinolone resistance in lab and clinical isolates (Poole, 2011). Although there are several additional chromosomally encoded efflux pumps that are able to recognize fluoroquinolones (e.g., MexHSI-OpmI, MexVW-OprM, the NorM ortholog; Morita et al., 1998; Li et al., 2003; Sekiya et al., 2003; He et al., 2004).

The formation of static biofilms increases when *P. aeruginosa* cells are incubated in the presence of sub-inhibitory concentrations of ciprofloxacin, tobramycin, or tetracycline, while no such sub-inhibitory effect is detected with members of other antibiotic classes, such as carbencillin, chloramphenicol, or polymyxin (Hoffman et al., 2005; Linares et al., 2006). However, in the presence of a sub-inhibitory concentration of ciprofloxacin (but not of tetracycline or tobramycin), *P. aeruginosa* shows a reduction in swimming and swarming, both of which are important systems of bacterial motility and probably related to the pathogenic process in CF patients (Linares et al., 2006).

Transcriptional responses of *P. aeruginosa* to sub-inhibitory and inhibitory concentrations of ciprofloxacin demonstrate the induction or repression of 100s of genes (Brazas and Hancock, 2005). These inhibitory concentrations of ciprofloxacin lead to sub-inhibitory levels of ciprofloxacin or ofloxacin enhance the frequency of mutation to carbapenem (especially meropenem) resistance (Tanamoto et al., 2008).

**RESPONSES TO AMINOGLYCOSES**

Aminoglycosides bind to the 30S ribosomal subunit and interfere with protein synthesis, causing mistranslation and ultimately cell death without lysis (Davis, 1987). APH(3′)-IIb, a chromosomal aminoglycoside phosphoryltransferase, is likely responsible for the general non-susceptibility of *P. aeruginosa* to kanamycin (Hachler et al., 1996), and APH(3′)-II predominates in clinical isolates resistant to kanamycin (Poole, 2005). Aminoglycoside uptake and subsequent action within bacterial cells is a complex process that involves LPS binding and outer-membrane permeation, cytoplasmic membrane traversal driven by membrane potential, and ribosome disruption, leading to the production of membrane-damaging mistranslated polypeptides (Davis, 1987; Krahn et al., 2012). Primary intrinsic and adaptive resistance to aminoglycosides is due to the MexXY multidrug efflux system in laboratory and clinical isolates (Morita et al., 2012b). The antagonism of aminoglycosides by divalent cations Mg2+ and Ca2+ is well documented in *P. aeruginosa*, and occurs via a process...
that requires the MexXY multidrug efflux system (Mao et al., 2001; Morita et al., 2012a). In wild-type P. aeruginosa cells, the MexXY efflux system is inducible by sub-inhibitory concentrations of aminoglycoside- and ribosome-targeting antimicrobials (e.g., chloramphenicol and tetracycline), a process shown to be involved in expression of the PA5471 gene product [recently renamed ArrC, for anti-repressor MexZ (Morita et al., 2006; Hay et al., 2013)] The PA5471 system also is inducible through interference via translation of the gene’s leader peptide, PA5471.1 (Morita et al., 2013). The PA5471 system also is inducible through interference in expression of the PA5471 gene product [recently renamed MexXY efflux pump genes, the greatest increases in gene expression levels in response to lethal concentrations of tobramycin involve a number of P. aeruginosa heat shock genes (e.g., hspG, hspA, groES, and araS; Kindrachuk et al., 2011). Under these conditions, the likely intracellular ATP-dependent AsrA protease is noteworthy because of its modest positive impact on aminoglycoside resistance (Kindrachuk et al., 2013). The Lon protease also is inducible by aminoglycosides (Marr et al., 2007). Sub-inhibitory concentrations of aminoglycosides, especially tobramycin, induce biofilm formation in P. aeruginosa (Hoffman et al., 2005). Notably, the aminoglycoside tobramycin also induces both swimming and swarming of P. aeruginosa (Linares et al., 2006). Also induced is the aminoglycoside response regulator (arr) gene, which is predicted to encode an inner membrane phosphodiesterase. The Arr substrate is cyclic di-guanosine monophosphate (c-di-GMP), a bacterial second messenger that regulates cell surface adhesiveness; c-di-GMP is essential for this induction and contributes to biofilm-specific aminoglycoside resistance (Hoffman et al., 2005).

RESPONSES TO 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). Very recently, the polymyxin mutant prevention concentrations (MPCs) for P. aeruginosa were shown to be very high (≥64 μg/ml), even for susceptible isolates (i.e., with minimum inhibitory concentration (MIC) ranges of 1–2 μg/ml; Choi and Ko, 2013). In the MPC studies, mutation to polymyxin resistance apparently can result from a single mutation (Choi and Ko, 2013).

The mechanism of action of polymyxins involves an initial stage of interaction with the lipid A of the LPS, leading to self-promoted uptake of polymyxins across the membrane, followed by cell death (Zhang et al., 2000; Fernandez et al., 2013). The most common mechanism of resistance to polymyxins has been shown to arise from modification of LPS lipid A with 4-amino-4-carboxinone, a process that has been seen both in vitro-selected mutants and in CF isolates; other unknown mechanisms remain under investigation (Miller et al., 2011; Poole, 2011; Moskowitz et al., 2012). This modification is carried out by the products of the arr-BCADTFE-ugd operon, otherwise known as pmfHIJKLm-ugd (McPhie et al., 2003; Yan et al., 2007). The two-component ParRS regulator leads to the induction of the LPS modification operon in response to sub-inhibitory concentrations of polymyxins B and colistin (Fernandez et al., 2010).

Approximately 0.5% of genes showed significantly altered expression upon exposure to sub-inhibitory concentration of colistin (Cummins et al., 2009), a frequency that is no less dramatic than that seen with the other anti-pseudomonas agents (e.g., cefotaxime, ciprofloxacin, and tobramycin) described above. The most striking alterations were up-regulation of the Pseudomonas quinolone signal (PQS) biosynthetic genes such as the pqxABCDN operon, the phenazine biosynthetic operon, and the arr operon (Cummins et al., 2009).

RESPONSES TO THE MAJOR HUMAN CATIONIC HOST DEFENSE PEPTIDE, LL-37

The major human cationic host defense peptide LL37 (a.k.a. hCAP-18, FALL-39, or cathelicidin antimicrobial peptide (CAMP)), a 37-amino-acid, 18-kDa peptide, is encoded by the cathelicidin gene (CAMP) and was originally identified in humans (Kosciuczuk et al., 2012). Sub-inhibitory concentration of LL-37 (1/4–1/128 of the MIC of 64 μg/ml) were shown to prevent biofilm formation by decreasing the attachment of P. aeruginosa cells, stimulating twitching motility, and influencing two major QS systems (Las and Rhl), leading to the down-regulation of genes essential for biofilm development (Overhage et al., 2008). Similar results were obtained using the bovine neutrophil peptide indolicidin, but no inhibitory effects on biofilm formation were detected using sub-inhibitory concentrations of the mouse peptide CRAMP (67%-identical with LL-37), polymyxin B, or the bovine bactericin homolog Bac2A (Overhage et al., 2008).

RESPONSES TO MACROLIDES

Macrolides such as erythromycin and azithromycin are widely used antibiotics that block translation by binding to the 50S ribosomal subunit. P. aeruginosa cells are intrinsically resistant to macrolides when tested in standard broth culture; for instance, the MIC of erythromycin for P. aeruginosa PAO1 is about 512 μg/ml in Mueller-Hinton broth (e.g., Morita et al., 2001; Buyck et al., 2012). Nonetheless, low-dose macrolides such as azithromycin are effective treatments in patients with chronic lung infections (Jaffe et al., 1998; Kudoh et al., 1998). Even at concentrations (e.g., 2 μg/ml of azithromycin) far below the MIC, macrolides inhibit the QS circuitry of P. aeruginosa strain PA01, leading to a reduction in virulence factor production (Tateda et al., 2001). Low-dose azithromycin shows bactericidal activity for P. aeruginosa biofilms, but selects for nfxB mutants, which overproduce the MexCD-OprJ efflux pump (Mulet et al., 2009). Notably, the AmpC β-lactamase produced by nfxB mutants is protective in biofilm growth, although over expression of the MexCD-OprJ pump is known to impair P. aeruginosa’s intrinsic resistance, which is dependent on the MexXY/MexAB-OprM efflux pump and the AmpC (Mulet et al., 2011).
Genome-wide approaches have revealed the QS antagonistic activities of azithromycin (e.g., inhibition of QS, reduction of virulence factor production, and strong induction of type-III secretion systems; Nalca et al., 2016; Skanderson et al., 2008). This modulation causes decreased expression of the genes encoding the MexAB-OprM efflux pump in P. aeruginosa (Sugimoto et al., 2008). Azithromycin inhibits expression of the small RNAs rsmY and rsmZ, a process that depends on the GacA/Rsm signal transduction pathway; this pathway is known to positively control P. aeruginosa QS (Perez-Martinez and Haas, 2011). Both effects of azithromycin on QS (quorum factor-dependent virulence factor production and cell death) require 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 also is demonstrated through mutations in 23S rRNA that confer azithromycin resistance in bacterial isolates of P. aeruginosa in chronically infected CF patients (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 and increased outer-membrane permeability (Bourtchuladze et al., 2012).

RESPONSES TO TETRACYCLINES AND CHLORAMPHENICOL

Tetracyclines are bacteriostatic antibiotics based on a hydroanthracene nucleus, which contains four fused rings. The class also includes glycylcyclines (e.g., tigecycline), a group of semisynthetic tetracycline derivatives containing a glycylamido substitution at position 9 (Yao and Moellering, 2011). Tetracyclines enter bacterial cells by a mechanism dependent on the ribosomal inhibitor-inducible PA5471 gene product (Dean et al., 2003; Morita et al., 2006). This effect is reminiscent of the induction of the MexEF-OprN efflux system (via the MexT activator) in response to chloramphenicol and nitrosoative stress (Fetar et al., 2011). Chloramphenicol is a nitro-aromatic antimicrobial and resembles a nitrosoative stress product (Fetar et al., 2011).

RESPONSE TO BIOCIDES

Pseudomonas aeruginosa also has been reported to contaminate disinfectants (e.g., chlorhexidine, benzalkonium, and triclosan) in hospital or other such environments, thereby compromising the disinfectant’s ability to reduce or eliminate bacterial contamination. Chlorhexidine and benzalkonium are cationic biguanides and a nitrogen-based quaternary ammonium compound, respectively. These biocides function by affecting the cell membrane, resulting in lysis and the loss of cytoplasmic material (Poole, 2002). The RND-type MexCD-OprJ multidrug efflux pump is induced by sub-inhibitory concentrations of disinfectants such as benzalkonium chloride or chlorhexidine (Morita et al., 2003), a process that is dependent upon the AlgU stress response factor (Fraud et al., 2008). Global transcriptome response to chlorhexidine includes up-regulation of the mexCD-oprJ and oprH-phoPQ operons and down-regulation of genes encoding proteins involved in membrane transport, oxidative phosphorylation, electron transport, and DNA repair (Nde et al., 2009). A P. aeruginosa variant highly adapted to benzalkonium showed increased resistance to fluoroquinolones, owing to mutations in the quinolone resistance-determining region of gyrA and to mutations in genes mexR and mfdA that encode repressors of mexAB-oprM and mexCD-oprJ, respectively (Mc Cay et al., 2010). Development of chlorhexidine-tolerant sub-populations in P. aeruginosa biofilms also is dependent on the mexCD-oprJ genes (Chuang et al., 2012).

Triclosan specifically inhibits fatty acid synthesis through inhibition of bacterial enoyl-acyl carrier protein reductase, although P. aeruginosa is intrinsically resistant to triclosan due to the structure of the pseudomonal FabV protein (a triclosan-resistant enoyl-acyl carrier protein reductase) and active efflux. This innate resistance stems from at least five RND efflux pumps, including MexAB-OprM (Mima et al., 2007; Zhu et al., 2010). In P. aeruginosa mutant cells lacking the mexAB-oprM genes, sub-inhibitory concentrations of triclosan lead to alterations in the expression of almost half the genome, with 28% of genes being significantly up-regulated and 16% being significantly down-regulated (Chauvin and Schweizer, 2012). QS-regulating genes are among the most strongly down-regulated, and surprisingly, iron homeostasis is completely blocked in triclosan-exposed cells, thus mimicking conditions with excess iron (Chauvin and Schweizer, 2012).

SUMMING UP AND FURTHER PROSPECTS

The primary biological responses of P. aeruginosa to sub-inhibitory concentrations of antimicrobials are summarized...
Table 1: Biological responses of P. aeruginosa exposed to various antimicrobials at levels below the inhibitory concentrations.

| Antimicrobials | Biological responses | References |
|----------------|---------------------|------------|
| β-lactams      | Induction of the AmpC β-lactamase(some β-lactams are inducers, but others are not) | Livermore (1995) |
| Carbapenems    | Formation of thicker and more robust biofilms | Bagge et al. (2004) |
| Ceftazidime    | Induction of mutagenesis and decreasing ciprofloxacin toxicity | Blazquez et al. (2006) |
| Fluoroquinolones | Induction of biofilm formation | Linares et al. (2006) |
|                | Reduction in swimming and swarming | Linares et al. (2006) |
|                | Induction of SOS response | Brazis and Hancock (2005) |
|                | Up-regulation of the bacteriophage-like pycnins | Brazis and Hancock (2005) |
|                | Shift from canonical DNA replication enzymes to inducible polymerases | Cirz et al. (2006) |
|                | Enhancement of mutation frequency to β-lactam resistance | Wolter et al. (2007), Tanimoto et al. (2008) |
| Protein synthesis inhibitors | Induction of the MexXY efflux system | Morita et al. (2012b) |
| Aminoglycosides | Induction of heat shock genes | Kindrachuk et al. (2011) |
|                | Induction of biofilm formation | Hoffman et al. (2005) |
|                | Induction of swimming and swarming | Linares et al. (2006) |
|                | Induction of the Lon protease | Marr et al. (2007) |
| Macrolides     | Quorum sensing antagonistic activity(reduction in virulence factor) | Tateeda et al. (2001) |
|                | Down-regulation of the MexAB-OprM pump | Nalca et al. (2006) |
| Tetrazycline   | Induction of biofilm formation | Linares et al. (2006) |
|                | Induction of the T3SS and cytotoxicity | Linares et al. (2006) |
| Chlormphenicol | Induction of the MexEF-OprN efflux pump | Fietal et al. (2011) |
| Polyoxins      | Modification of LPS lipid A with 4-amino-L-arabinose | Fernandez et al. (2010) |
|                | Up-regulation of the PQS biosynthetic genes | Cummins et al. (2009) |
| Chlorhexidine  | Induction of the MexCD-OprJ efflux pump | Morita et al. (2003) |
|                | Induction of the oprH-phoPQ operon | Nide et al. (2009) |

In Table 1, Interestingly, clinically useful anti-pseudomonas agents (carbapenems, fluoroquinolones, and aminoglycosides) are known to efficiently kill P. aeruginosa planktonic cells when used properly, but lead to more severe biofilm development upon exposure at sub-inhibitory concentrations. This pattern means that optimization of anti-pseudomonas chemotherapy is critical, in the absence of such optimization, chemotherapy fails to treat the infection and readily selects multidrug-resistant P. aeruginosa. By contrast, macrolides (which are not effective against P. aeruginosa planktonic cells because of the planktonic cells’ intrinsic resistance) provide activity that is antagonistic to QS, thereby reducing pseudomonal virulence. These findings regarding responses to antimicrobial agents suggest a route toward conquering P. aeruginosa infections. For example, macrolides have been shown to augment the in vitro activity of anti-pseudomonas agents against biofilms (Lutz et al., 2012).

We are aware of potential molecular targets for novel anti-pseudomonas agents, including essential genes (Morita et al., 2010). Novel class anti-pseudomonas agents should be expected to minimize a severe situation in which few agents are effective against these organisms. However, it is a much more difficult task to develop novel class antimicrobials for P. aeruginosa because of the presence of low membrane permeability and the RND multidrug efflux pumps. In addition this organism has the ability to adapt to various stresses, including sub-inhibitory antimicrobial exposure, by recruiting antimicrobial resistance mechanisms, notably that of RND efflux systems such as the MexXY system. While many laboratories are currently screening, so far no efflux pump inhibitors have been made available for clinical settings. Screening for novel antibacterial agents, including efflux pump inhibitors, is currently in progress in many laboratories, including our own.

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REFERENCES

Aminov, R. I. (2013). Biotic acts of antibiotics. Front. Microbiol. 4:241. doi: 10.3389/fmicb.2013.00241
Chuanchuen, R., and Schweizer, H. P. (2012). Global transcriptional responses to triclosan exposure in clinical isolates. Antimicrob. Agents Chemother. 56, 354–362. doi: 10.1128/AAC.002519-12

Chiou, W. C., Pamp, S. J., Nilsson, M., Givskov, M., and Tølker-Nielsen, T. (2013). Characterization of the polymyxin B resistome of Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 57, 110–119. doi: 10.1128/AAC.00830-10

Chong, W. C., Pamp, S. J., Nilsson, M., Givskov, M., and Tolk-Nielsen, T. (2012). The metabolically active subpopulation in Pseudomonas aeruginosa biofilms releases exposure to membrane-targeting antimicrobials via distinct molecular mechanisms. JEMS Immunol. Med. Microbiol. 65, 245–256. doi: 10.1111/j.1574-695X.2012.00930.x

Choi, M. J., and Ko, K. S. (2013). Mutant prevention concentrations of colistin for Acinetobacter baumannii. Pseudomonas aeruginosa and Escherichia coli: clinical isolates. J. Antimicrob. Chemother. 69, 275–277. doi: 10.1093/jac/dkt353

Choquet, B., and Schweizer, H. P. (2012). Global transcriptional responses to triclosan exposure in Pseudomonas aeruginosa. Int. J. Antimicrob. Agents 40, 114–122. doi: 10.1016/j.ijantimicag.2012.04.008

Cin, B. T., O’Neill, R. M., Hammond, J. A., S. R., and Remberg, E. F. (2006). Defining the Pseudomonas aeruginosa SOS response and its role in the global response to the antibiotic ciprofloxacin. J. Bacteriol. 188, 7105–7110. doi: 10.1128/JB.00830-07

Ciminero, J., Jeon, F. J., Brescia, C., Mouly, M. J. I., and Oltra, F. (2009). Sub- lethal exposure to beta-lactam antibiotics induce the Pseudomonas quinolone signal in Pseudomonas aeruginosa. Microbiology 155, 2026–2037. doi: 10.1099/mic.0.2008/002483-0

Dennis, D. B. (1987). Mechanism of bactericidal action of aminoglycosides. Microbiol. Rev. 51, 341–350.

Dias, C. R., Vissali, M. A., Projan, S. J., Sum, P. E., and Bradford, P. A. (2001). Efflux-mediated resistance to tigecycline (GAR-936) in Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 45, 2972–2978. doi: 10.1128/AAC.45.7.2972-2978.2001

Fernandez, L., Gooderham, W. J., Bains, M., McPhee, J. B., Wiegand, I., and Hancock, R. E. (2010). Adaptive resistance to the “last hope” antibiotics polymyxin B and tigecycline (GAR-936) in Pseudomonas aeruginosa PAO1. Antimicrob. Agents Chemother. 54, 3572–3582. doi: 10.1128/AAC.00582-10

Fernandez, L., Gooderham, W. J., Bains, M., McPhee, J. B., Wiegand, I., and Hancock, R. E. (2010). The type III secretion system of Pseudomonas aeruginosa: involvement in chlorhexidine resistance and induction by membrane-damaging agents dependent upon the MexXY/OprF multidrug efflux system of Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 54, 3572–3582. doi: 10.1128/AAC.00582-10
Mulet, X., Moya, B., Juan, C., Macia, M. D., Perez, J. L., Blazquez, J., et al. (2011). Interplay between chromosomal beta lactamases and mexA-mexB-oprM efflux sys-
tem in intrinsic resistance to beta lactams in Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 45, 400–402.

Mc Cor, E. H., Ocampo-Sousa, A. A., and Fleming, G. T. (2010). Effect of subin-
hbitory concentrations of benzalkonium chloride on the competitiveness of Pseudomonas aeruginosa grown in continuous culture. Microbiology 156, 30–38. doi: 10.1099/mic.0.029753-0

McPhee, J. B., Lewenza, S., and Hancock, R. E. (2003). Cationic antimicro-
bial peptides activate a two-compartment regulatory system, PamA-PamB, that regulates resistance to polymyxin B and cationic antimicrobial peptides in Pseudomonas aeruginosa: Mut. Microbiol. 50, 203–217. doi: 10.1111/j.1365-2958.2000.02587.x

Miller, A. K., Brannon, M. K., Sterner, L., Johnson, H. K., Selgrade, S. E., Miller, S. I., et al. (2011). PmrA mutations promote lipid A modification and polymyxin resistance of Pseudomonas aeruginosa found in colonized cystic fibrosis patients. Antimicrob. Agents Chemother. 55, 5761–5769. doi: 10.1128/AAC.00181-11

Mima, T., Joshua, S., Gomez-Escalada, M., and Schweizer, H. P. (2007). Identification and characterization of TraEC-OprM, a tRNA efflux pump of Pseudomonas aeruginosa requiring two membrane fusion proteins. J. Bacteriol. 179, 7609–7609. doi: 10.1128/JB.00090-07

Motta, T., Gilmour, C., Metcalf, D., and Poole, K. (2009). Translational control of the antibiotic inducibility of the PmrA1 gene required for mexAB-mexCD efflux gene expression in Pseudomonas aeruginosa. J. Bacteriol. 191, 4905–4905. doi: 10.1128/JB.00115-08

Motta, T., Kurihara, N., Mima, T., Mima, T., and Tsuchiya, T. (2005). Roles of MexXY-mexMK-fpr-mexF multidrug efflux pumps in intrinsic resistance of Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 49, 27–52. doi: 10.1128/AAC.49.1.27-52.2005

Motta, T., Kodama, K., Shota, S., Mima, T., Katoaka, A., Mima, T., et al. (1998). NovA, a peptides multidrug efflux protein, of V. parahaemolyticus and its homolog in Escherichia coli. Antimicrob. Agents Chemother. 42, 1778–1782.

Motta, T., Murata, T., Mima, T., Shota, S., Kuroda, T., Mima, T., et al. (2002). Induction of mexA-mexB OprM by a multidrug efflux pump is dismantled in a wild-type Pseudomonas aeruginosa PAO1. J. Antimicrob. Chemother. 51, 991–994. doi: 10.1093/jac/dkg177

Motta, T., Nartia, S., Tomida, J., Tsuchiya, H., and Kawamura, Y. (2010). Appli-
cation of an inducible system to engineer unmarked conditional mutants of essential genes of Pseudomonas aeruginosa. J. Microbiol. Methods 82, 205–215. doi: 10.1016/j.mimet.2009.06.001

Motta, T., Sobei, M. I., and Poole, K. (2006). Antibiotic inducibility of the MexXY multidrug efflux system of Pseudomonas aeruginosa: involvement of the antibiotic-inducible PA5471 gene product. J. Bacteriol. 188, 1547–1555. doi: 10.1128/JB.188.5.1547-1555.2006

Motta, T., Tomida, J., and Kawamura, Y. (2012a). MexXY multidrug efflux system of Pseudomonas aeruginosa: ATP-driven exchange of multiple hydrophobic substrates. J. Antimicrob. Chemother. 51, 1071–1083. doi: 10.1093/jac/dks380

Motta, T., Tomida, J., and Kawamura, Y. (2012b). Primary mechanisms medi-
at ing aminoglycoside resistance in the multidrug-resistant mexXY and MexAB mutants. J. Bacteriol. 194, 2080–2080. doi: 10.1128/JB.00850-07

Mima, T., Joshi, S., Gomez-Escalada, M., and Schweizer, H. P. (2007). Identification and characterization of TraEC-OprM, a tRNA efflux pump of Pseudomonas aeruginosa requiring two membrane fusion proteins. J. Bacteriol. 191, 7609–7609. doi: 10.1128/JB.00090-07

Motta, T., Gilmour, C., Metcalf, D., and Poole, K. (2009). Translational control of the antibiotic inducibility of the PmrA1 gene required for mexAB-mexCD efflux gene expression in Pseudomonas aeruginosa. J. Bacteriol. 191, 4905–4905. doi: 10.1128/JB.00115-08

Motta, T., Kurihara, N., Mima, T., Mima, T., and Tsuchiya, T. (2005). Roles of MexXY-mexMK-fpr-mexF multidrug efflux pumps in intrinsic resistance of Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 49, 27–52. doi: 10.1128/AAC.49.1.27-52.2005

Motta, T., Kodama, K., Shota, S., Mima, T., Katoaka, A., Mima, T., et al. (1998). NovA, a peptides multidrug efflux protein, of V. parahaemolyticus and its homolog in Escherichia coli. Antimicrob. Agents Chemother. 42, 1778–1782.

Motta, T., Murata, T., Mima, T., Shota, S., Kuroda, T., Mima, T., et al. (2002). Induction of mexA-mexB OprM by a multidrug efflux pump is dismantled in a wild-type Pseudomonas aeruginosa PAO1. J. Antimicrob. Chemother. 51, 991–994. doi: 10.1093/jac/dkg177

Motta, T., Nartia, S., Tomida, J., Tsuchiya, H., and Kawamura, Y. (2010). Appli-
cation of an inducible system to engineer unmarked conditional mutants of essential genes of Pseudomonas aeruginosa. J. Microbiol. Methods 82, 205–215. doi: 10.1016/j.mimet.2009.06.001

Motta, T., Sobei, M. I., and Poole, K. (2006). Antibiotic inducibility of the MexXY multidrug efflux system of Pseudomonas aeruginosa: involvement of the antibiotic-inducible PA5471 gene product. J. Bacteriol. 188, 1547–1555. doi: 10.1128/JB.188.5.1547-1555.2006

Motta, T., Tomida, J., and Kawamura, Y. (2012a). MexXY multidrug efflux system of Pseudomonas aeruginosa: ATP-driven exchange of multiple hydrophobic substrates. J. Antimicrob. Chemother. 51, 1071–1083. doi: 10.1093/jac/dks380

Motta, T., Tomida, J., and Kawamura, Y. (2012b). Primary mechanisms medi-
Zhu, L., Lin, J., Ma, J., Cronan, J. E., and Wang, H. (2010). Triclosan resistance of Pseudomonas aeruginosa PAO1 is due to FabV, a triclosan-resistant enoyl-acyl carrier protein reductase. Antimicrob. Agents Chemother. 54, 689–698. doi: 10.1128/AAC.01152-09

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