Antimicrobial Resistance Mechanisms in *Pseudomonas aeruginosa*

NICHOLAS M MOORE, MARIBETH L FLAWS

Learning Objectives:
Upon reading this article, the reader will be able to:

1. Describe the various resistance mechanisms utilized by *Pseudomonas aeruginosa*.
2. Differentiate between acquired and innate resistance mechanisms in *P. aeruginosa*.
3. List the antimicrobial-modifying enzymes expressed by *P. aeruginosa*.
4. Name the mechanism used by *P. aeruginosa* to resist the activity of each of the antimicrobial agents that are used in treatment.

ABBREVIATIONS: NHSN = National Healthcare Safety Network; HAI = healthcare-associated infections; OprF = outer membrane protein F.

INDEX TERMS: *Pseudomonas aeruginosa,* antimicrobial resistance, membrane efflux.

Clin Lab Sci 2011;24(1):47

***Nicholas M. Moore, MS, MLS(ASCP)**, Rush University, Department of Medical Laboratory Science, Chicago, IL*

***Maribeth L. Flaws, PhD, SM(ASCP)SI**, Rush University, Department of Medical Laboratory Science, Chicago, IL*

***Address for Correspondence:** Nicholas M. Moore, MS, MLS (ASCP), Department of Medical Laboratory Science, 600 S. Paulina St., Suite 1014, Chicago, IL 60612, 312-942-2111, Fax: (312) 942-6464, Nicholas_Moore@rush.edu*

Pseudomonas aeruginosa is one of the leading causes of healthcare-associated infections (HAI). In the more severe infections such as bacteremia and pneumonia, mortality rates are high, and the infection is often difficult to treat because there are limited drugs with anti-pseudomonal activity. Infections caused by *P. aeruginosa* may be treated with antimicrobial agents from three major groups based on mechanism of action: aminoglycosides (interference with protein synthesis) such as tobramycin or amikacin; beta-lactams (inhibition of cell wall synthesis) such as piperacillin, ticarcillin, 3rd and 4th generation cephalosporins (ceftazidime and cefepime), and carbapenems like imipenem or meropenem; or fluoroquinolones (ciprofloxacin) (interference with nucleic acid replication). In 2005, the National Healthcare Safety Network (NHSN) began collecting, summarizing and reporting data on antimicrobial-resistant pathogens that cause HAI. As described in their 2008 report, HAI pose an "ongoing and increasing challenge to hospitals, both in the clinical treatment of patients and in the prevention of the cross-transmission of these problematic pathogens".[1]

As reported by the NHSN, increases in antimicrobial resistance have significantly limited the number of available treatment options.[2-4] Production of new classes of antibiotics has stalled in the last two decades, and novel agents with activity against *P. aeruginosa* will not be available in the foreseeable future, thus continuous surveillance of the development of resistance to current therapeutic agents is vital as is the appropriate utilization of antimicrobial agents to minimize the development of resistance.

To investigate the development of resistance to beta-lactam antibiotics, one group followed 132 intensive care unit (ICU) patients over a three-year period.[5] They tracked the antibiogram of *P. aeruginosa* isolated from these patients over time along with the type of beta-lactam the patient was prescribed. They found that the *P. aeruginosa* isolated from patients on imipenem and piperacillin/tazobactam became resistant to those drugs

more than when patients were prescribed amoxicillin-clavulanic acid, ceftazidime, or cefepime.[5] Obritsch and colleagues analyzed the resistance of *P. aeruginosa* to antimicrobial agents from data collected from 1993-2002 in the Intensive Care Unit Surveillance Study (ISS) database.[6] They found that *P. aeruginosa* became significantly more resistant to ciprofloxacin, imipenem, tobramycin and aztreonam over the ten year period that was studied. In addition, resistance to multiple drugs also increased. An interesting study performed by Kallel et al[7] tracked the usage of antimicrobial agents and resistance of *P. aeruginosa* to those agents. They found that when imipenem and ciprofloxacin were given to ICU patients, *P. aeruginosa* showed increasing resistance to imipenem and ciprofloxacin. On the other hand, when the usage of imipenem and ciprofloxacin decreased, the resistance of *P. aeruginosa* to those drugs also decreased. These studies clearly demonstrate that *P. aeruginosa* is becoming increasingly more resistant to antimicrobial agents.

But how do bacteria, specifically *P. aeruginosa*, develop antibiotic resistance? Resistance occurs through a variety of mechanisms, such as: 1) intrinsic resistance to antimicrobial agents via a large selection of genetically-encoded resistance mechanisms; 2) mutations in chromosomal genes; or 3) acquisition of additional resistance mechanisms from other bacterial organisms in the form of plasmid-encoded genes.[8]

The genome of *P. aeruginosa* is among the largest amongst bacteria with 5567 genes encoded in 6.26 Mbp as compared to *Escherichia coli* K12 that has 4.64 Mbp encoding 4279 genes and *Haemophilus influenzae* Rd that has 1714 genes in 1.83 Mbp of DNA.[9] This comparatively large genomic size allows for greater genetic capacity, especially when only about 1500 genes are needed for the organism to grow and replicate, and presumably accounts for its highly adaptable nature, including its ability to develop resistance to antibiotics.[9]

Intrinsic or Inherent Resistance

P. aeruginosa is inherently resistant to certain antimicrobial agents by three different mechanisms including decreased permeability of the outer cell membrane preventing the agent from getting into the cell, the use of mechanical efflux pumps which actively pump the antimicrobial agent out of the cell and the production of enzymes that degrade the antimicrobial agent destroying its activity.

Permeability of outer cell membrane

Although the drugs typically used to treat *P. aeruginosa* infections have different mechanisms of actions, they all must first cross the bacterial cell wall in order to reach their target. All gram-negative bacteria have an outer cell membrane as a component of its cell wall that naturally prevents large, hydrophilic molecules from passing through it. In order to get inside the cell, these molecules must pass through porins, which are protein channels that span the outer membrane. With regard to antimicrobial agents, beta-lactams and quinolones must diffuse through porin channels in the outer membrane of the gram negative cell wall, but aminoglycosides and colistin interact with lipopolysaccharide on the outside of the outer membrane changing the permeability of the membrane so that they can pass through the membrane.

There are two major classes of porin channels produced by bacteria: general and specific. General porins will allow almost any hydrophilic molecule to pass through it whereas specific porins have binding sites to which certain molecules will bind orienting the molecule so that it passes through the porin in the most energy-efficient manner.[10] The specific porins are particularly important for moving certain molecules into the cell especially when that molecule is in short supply, but just about any molecule can pass through a specific porin similarly to a general porin. Most gram negative organisms have lots of general porins and relatively few specific porins, but *P. aeruginosa* is opposite in that it has mainly specific porins in its outer membrane.[10]

P. aeruginosa produces several different porin channels and these porins contribute to the inherent resistance of the organism to antimicrobial agents. The major general porin of *P. aeruginosa* is outer membrane protein F (OprF). While most molecules can pass through OprF, the channels are often very narrow and practically not many molecules pass through. There have been mutant strains lacking OprF reported in the literature, but loss of OprF has not been found to be a major cause of antibiotic resistance.[11] Specific porins found in *P. aeruginosa* along with their substrate include:

- OprB specific for glucose
- OprP specific for phosphate
- OprO specific for polyphosphate and
- OprD specific for positively charged amino acids such as lysine.

Imipenem and the other carbapenems cross the outer membrane through OprD, although other beta-lactams cannot use this porin to cross the outer membrane.[3] Loss of this porin has been associated with increased resistance to carbapenems, but as expected, not to other beta-lactams.[3] In fact, the minimum inhibitory concentration of imipenem increases from 1–2 mg/L to 8–32 mg/L when OprD expression is lost.[9] OprD must not be the only porin through which the carbapenems cross, however, because the loss of OprD does not affect the susceptibility of the organism to meropenem.[3]

Unlike the beta-lactams and quinolones, which utilize porin channels to cross the outer membrane to get to their target, the aminoglycosides bind to the lipopolysaccharide (LPS) on the outer membrane surface, increasing the permeability of the membrane so that the aminoglycoside can get to the cytoplasmic membrane. OprH is an outer membrane protein that protects LPS from being bound by the aminoglycosides. Aminoglycoside resistance due to the overexpression of OprH has been observed in laboratory strains but not in many clinical isolates.[9] Resistance of *P. aeruginosa* to aminoglycosides is due to other mechanisms which will be discussed below.

Efflux pumps

The second mechanism by which *P. aeruginosa* exerts resistance to antimicrobial agents is by actively pumping the drug out of the cell through efflux pumps. The presence of efflux pumps in *P. aeruginosa* was first reported in 1993.[12] Many clinically relevant gram-negative organisms possess efflux pumps. The efflux pumps of *P. aeruginosa* are multi-drug resistance (MDR) transporters that use a proton motive force to move the drug out of the cell. In particular, the MDR transporters of *P. aeruginosa* are part of the resistance-nodulation-cell division (RND) family.[13] The efflux pumps are comprised of three proteins: an RND exporter protein embedded in the cytoplasmic membrane that is an energy-dependent pump, a membrane fusion protein in the periplasmic space that links the RND exporter protein to the third protein, an outer membrane porin.[9] The RND exporter protein and the membrane fusion protein are named multidrug efflux (Mex) along with a letter, *e.g.* MexA and MexB. The outer membrane proteins are called Opr's like the porins described above used by the beta-lactams to enter the cell.[14] *P. aeruginosa* has twelve RND efflux systems, but only half of them have been characterized.[14] The efflux pumps that remove antimicrobial agents along with some of their substrates are:[15]

- MexAB-OprM: Beta-lactams, fluoroquinolones
- MexXY-OprM: Fluoroquinolones, beta-lactams, aminoglycosides
- MexCD-OrpJ: Beta-lactams, fluoroquinolones
- MexEF-OprN: Fluoroquinolones
- MexJK-OprM: Tetracycline, erythromycin
- MexPQ-OpmE: Fluoroquinolones
- MexGHI-OpmD: Fluoroquinolones

Most antimicrobial agents are pumped out by these efflux pumps. Only the polymyxins are not removed from the cell via efflux pumps.

Antimicrobial modifying enzymes

The production of enzymes that degrade antimicrobial agents is the third way *P. aeruginosa* resists the inhibitory effects of antimicrobial agents. Most of these enzymes are encoded on plasmids that are acquired by *P. aeruginosa* from the environment and will be discussed later. *AmpC* is one antimicrobial-degrading enzyme that is encoded in the genome. *AmpC* encodes a beta-lactamase. Beta-lactamases are enzymes that cleave the beta-lactam ring, opening up the structure and destroying the ability of the beta-lactam to bind to its target. The *Amp*C of *P. aeruginosa* is an inducible cephalosporinase that confers resistance to all beta-lactams except the carbapenems.

Acquired Resistance

P. aeruginosa is inherently resistant to antimicrobial agents because of restricted access of extracellular antimicrobial agents to intracellular targets, active efflux of antimicrobial agents out of the cell and production of

enzymes that degrade antimicrobial agents. *P. aeruginosa* has also been observed to acquire resistance to antimicrobial agents either through mutations to chromosomal genes or through the acquisition of plasmids encoding genes conferring resistance.

Mutations to chromosomal genes

With regard to mutations in chromosomal genes, several genes have been identified that when mutated confer resistance. First of all, mutation of the *gyrA* gene changes the structure of DNA gyrase which is the target of the quinolones.[9] The structure of the 30S subunit of the ribosome can also be changed by mutation resulting in resistance to streptomycin but not to other anti-pseudomonal aminoglycosides. The gene encoding the penicillin-binding protein when mutated confers resistance to beta-lactams.[9] Mutations in genes that regulate the expression of genes involved in the resistance of the organism to antimicrobial agents can also increase the resistance of the organism. For example, mutations in *ampR*, the gene that regulates the expression of *ampC*, results in the overexpression of *ampC* and thereby increases resistance to beta-lactams.[15]

Acquisition of resistance genes on plasmids

Many different resistance genes are encoded on plasmids that have been shown to be acquired by *P. aeruginosa*. These genes either confer resistance to beta-lactams or to the aminoglycosides. Several types of beta-lactamases with differing substrate specificity have been identified in many bacterial organisms. *P. aeruginosa* has been shown to produce the following beta-lactamases:[15]

- Class A serine beta-lactamases (PSE, CARB, and TEM)
- Class A extended-spectrum beta-lactamases (ESBL) (TEM, SHV, CTX-M, PER, VEB, GES, and IBC)
- Class D OXA-type ESBLs
- Class B metallo-beta-lactamases that degrade all beta-lactams including the carbapenems (IMP, VIM, SPM, and GIM) and
- Class A Carbapenemases (KPC) that degrade the carbapenems.

P. aeruginosa also acquires plasmids encoding aminoglycoside-modifying enzymes.[16] The aminoglyco-side-modifying enzymes found in *P. aeruginosa* either phosphorylate (aminoglycoside phosphoryltransferase (APH)), acetylate (aminoglycoside acetyltransferase (AAC)), or adenylate (aminoglycoside nucleotidyl-transferase (ANT)) the aminoglycoside. *P. aeruginosa* can express one or more aminoglycoside modifying enzymes increasing the spectrum of resistance with increasing expression of enzymes.[16] The major aminoglycoside modifying enzymes and their substrates are:

- AAC(6')-I confers resistance to tobramycin, netilmicin and amikacin
- AAC(6')-II confers resistance to gentamicin, tobramycin and netilmicin
- AAC(3)-I confers resistance to gentamicin
- AAC(3)-II confers resistance to gentamicin, tobramycin and netilmicin
- AAC(6')-I confers resistance to tobramycin, netilmicin and amikacin.
- ANT(2')-I confers resistance to gentamicin and tobramycin and
- APH(3')-VI confers resistance to amikacin and isepamicin.

As evident from above, *P. aeruginosa* possesses a wide repertoire of mechanisms allowing it to be resistant to most anti-pseudomonal drugs. Resistance can be chromosomally-mediated as in expression of efflux pumps or acquired through mutation of chromosomal genes or expression of plasmid-encoded resistance genes. Rising rates of nosocomial infections, increasing rates of antibiotic resistance and no new drugs with activity against *P. aeruginosa* scheduled to be produced in the future has increased the concern about treatment measures for infections caused by *P. aeruginosa*. The next article in this series will discuss the treatment modalities that are currently recommended for *P. aeruginosa*.

REFERENCES

1. Hidron AI, Edwards JR, Patel J, *et al.* Antimicrobial-resistant pathogens associated with healthcare-associated infections: Annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol. 2008;29:996-1011.

2. Levin AS, Barone AA, Penco J, *et al*. Intravenous colistin as therapy for nosocomial infections caused by multi-drug resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* Clin Infect Dis 1999;28:1008-11.
3. Livermore DM. Of *Pseudomonas*, porins, pumps and carbapenems. J Antimicrob Chemother 2001;47:247-50.
4. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare. Clin Infect Dis 2002;34:634-40.
5. Georges B, Conil J, Dubouix A, *et al*. Risk of emergence of *Pseudomonas aeruginosa* resistance to beta-lactam antibiotics in intensive care units. Crit Care Med 2006;34:1636-41.
6. Obritsch MD, Fish DN, MacLaren R, *et al*. National Surveillance of Antimicrobial Resistance in *Pseudomonas aeruginosa* Isolates Obtained from Intensive Care Unit Patients from 1993 to 2002. Antimicrob Agents and Chemo 2004;48:4606-10.
7. Kallel H, Mahjoubi F, Dammak H, et al. Correlation between antibiotic use and changes in susceptibility patterns of *Pseudomonas aeruginosa* in a medical-surgical intensive care unit. Indian J Crit Care Med 2008;12:18-23.
8. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. *Am J Med* 2006;119(Suppl 1):S3-S10.
9. Lambert PA. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. J R Soc Med 2002;95:22-6.
10. Tamber S, Ochs, MM, Hancock REW. Role of the novel OprD family of porins in nutrient uptake in *Pseudomonas aeruginosa*. J Bacteriol 2006;188:45-54.
11. Hancock REW, Brinkman F. Function of *Pseudomonas* porins in uptake and efflux. Annu. Rev. Microbiol. 2002;56:17–38.
12. Poole K, Krebes K, McNally C, Neshat S. Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. J Bacteriol 1993;175:7363-72.
13. Mazurkiewicz P, Driessen AJM, Konings WN. What do proton motive force driven multidrug resistance transporters have in common? Curr Issues Mol Biol 2005;7:7-22.
14. Schweizer HP. Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeruginosa* and related bacteria: unanswered questions. Genet Mol Res 2003;2:48-62.
15. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Micro Rev 2009;22:582-610.
16. Poole K. Aminoglycoside resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2005;49:479-87.

The Focus section seeks to publish relevant and timely continuing education for clinical laboratory practitioners. Section editors, topics, and authors are selected in advance to cover current areas of interest in each discipline. Readers can obtain continuing education credit (CE) through P.A.C.E.® by completing the continuing education registration form, recording answers to the examination, and mailing a photocopy of it with the appropriate fee to the address designated on the form. Suggestions for future Focus topics and authors, and manuscripts appropriate for CE credit are encouraged. Direct all inquiries to the Clin Lab Sci Editorial Office, Westminster Publishers, 315 Westminster Court, Brandon MS 39047. (601) 214-5028, (202) 315-5843 (fax). westminsterpublishers@comcast.net.