

Mechanisms of Multidrug Resistance in *Acinetobacter* Species and *Pseudomonas aeruginosa*

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Acinetobacter species and *Pseudomonas aeruginosa* are noted for their intrinsic resistance to antibiotics and for their ability to acquire genes encoding resistance determinants. Foremost among the mechanisms of resistance in both of these pathogens is the production of β -lactamases and aminoglycoside-modifying enzymes. Additionally, diminished expression of outer membrane proteins, mutations in topoisomerases, and up-regulation of efflux pumps play an important part in antibiotic resistance. Unfortunately, the accumulation of multiple mechanisms of resistance leads to the development of multiply resistant or even “panresistant” strains.

The emergence and spread of antibiotic-resistant bacteria causing infection is of great concern to clinicians. Since the seminal description of a penicillin-inactivating enzyme in *Escherichia coli*, the fierce 75-year struggle against these bacteria has been aptly referred to as an “unwinnable war” (Wellcome Trust Web site [available at: <http://www.wellcome.ac.uk>]) [1]. Recent clinical attention has focused on the increasing frequency of non-lactose-fermenting gram-negative pathogens responsible for hospital-acquired infections [2]. In this group, *Acinetobacter* species and *Pseudomonas aeruginosa* are emerging as pathogens that frequently cause infections in patients in intensive care units [3]. In both genera of bacteria, resistance to multiple classes of antibiotics seriously compromises our ability to treat patients who are infected with these pathogens. In many instances, there are perilously few antibiotic choices. Hence, for the immunocompromised host, timely institution of effective therapy is a matter of survival. In this review, we highlight the molecular basis for antibiotic resistance in *Acinetobacter* species and *P. aeruginosa* (table 1). Once these mechanisms are understood, clinicians may seek to devise interventions that will translate into a “truce” in this inexorable struggle.

ANTIBIOTIC RESISTANCE IN *ACINETOBACTER BAUMANNII*

***AmpC* cephalosporinases.** Numerous β -lactamases have been described in *A. baumannii*. The chromosomally encoded cephalosporinase (AmpC type) is common to all strains of *A. baumannii*. Evidence has accumulated that these cephalosporinases are genetically related [4–12]. Furthermore, phylogenetic analysis suggests that this cephalosporinase should be placed in a unique subgroup among the class C β -lactamases [13]. To date, there has been no evidence to indicate that the chromosomal cephalosporinase is inducible [13]. Recently, insertion sequences (ISs) have been found that increase production of the chromosomal β -lactamase of *A. baumannii* (a 1200-bp sequence described by Corvec et al. [7], IS_{Abal} [14], and IS1135 [15]). In a study by Segal et al. [14], a cephalosporinase of *A. baumannii* is transcribed from a promoter contained within a putative IS element in *Acinetobacter* species.

Other β -lactamases. In addition to the class C cephalosporinase discussed above, other β -lactamases have been reported in *A. baumannii*. These include the TEM-1 type [6, 16], SHV type [17, 18], CTX-M type [19], PER-1 [20–22], and VEB-1 [23, 24] β -lactamases. Although they are important, it is difficult to assess their impact on resistance in the presence of the AmpC cephalosporinase.

Serine and metallo- β -lactamases (carbapenemases). The most problematic recent occurrence is the emergence of numerous OXA enzymes in *A. baumannii*

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Table 1. Mechanisms of antibiotic resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*.

Mechanism	<i>Acinetobacter</i> species	<i>P. aeruginosa</i>
β-Lactamases		
AmpC cephalosporinase	+	–
Inducible	–	+
TEM	+	+
SHV	+	+
CTX-M	+	–
PER	+	+
VEB	+	+
OXA ^a	+	+
IMP	+	+
VIM	+	+
SPM	–	+
GIM	–	+
PSE	–	+
GES	–	+
IBC	–	+
OMP changes	+	+
AMEs		
Adenylating	+	+
Phosphorylating	+	+
Acetylating	+	+
Topoisomerase mutations		
<i>gyrA</i>	+	+
<i>parC</i>	+	+
Efflux pumps	+	+
Mobile genetic elements	+	+
Integrans	+	+
Membrane changes and resistance to polymyxin	–	+

NOTE. +, present; –, absent; AME, aminoglycoside-modifying enzyme; ESBL, extended-spectrum β -lactamase; OMP, outer membrane protein.

^a OXA β -lactamases are of 2 types: carbapenemases (found in *Acinetobacter* species) and ESBL-type (found in *P. aeruginosa*).

that confer β -lactam resistance [25]. Of note, the use of carbapenems to treat *A. baumannii* infection has also resulted in outbreaks of infection with carbapenem-resistant *Acinetobacter* species [26–28]. The first description of a serine carbapenemase in *A. baumannii* was ARI-1 (OXA-23), a clinical isolate from a blood culture at the Royal Infirmary in Edinburgh, Scotland, in 1985 [29]. Although OXA carbapenemases may not robustly hydrolyze imipenem, their presence in an organism that may have an IS element that acts as a promoter can result in imipenem resistance [30].

Many OXA β -lactamases are found as part of integrans [31–34]. Integrans are genetic elements of variable length that contain a 5' conserved integrase gene (*int*), gene cassettes, and an integration site for the gene cassette, *attI*. Three main classes of integrans have been described in *Acinetobacter*. In the class 1 integrans, the most common in *Acinetobacter* species, the 3'

conserved segment may include multiple open reading frames; *qacE Δ 1*, a deletion derivative of the antiseptic resistance gene *qacE*; and *sul1*, a sulfonamide resistance gene (figure 1).

Two major metallo- β -lactamases have been reported in *A. baumannii*: IMP and VIM type. VIM-2 β -lactamases detected in *A. baumannii* isolates from Korea confer significant levels of resistance to carbapenems [35–37]. This *bla*_{VIM-2} gene is located on 2 newly described integrans (class I integrans *In105* and *In106*) [35]. IMP type metallo- β -lactamases (IMP-1, -2, -5, -6, -11) are also being reported with increasing frequency [35, 37, 38]. Walsh et al. [37] summarize the genetic environment, classification, and biochemistry of this emerging threat and point out that many metallo- β -lactamases are found in class 1 integrans that are part of transposons (see below).

Outer membrane protein (OMP [*porin*]) changes. By reduction of transport into the periplasmic space via changes in porins or OMPs, access to penicillin-binding proteins is reduced. With less β -lactam entering the periplasmic space, the weak enzymatic activity of the β -lactamase is amplified. Many outbreaks of infection with imipenem-resistant *A. baumannii* are due to porin loss. Quale et al. [39] found that carbapenem-resistant isolates of *A. baumannii* had reduced expression of 47-, 44-, and 37-kDa OMPs. Similarly, Clark [40] found decreased expression of a 33- to 36-kDa protein in 2 imipenem-resistant strains of *A. baumannii* (A-1 and A-24). In a study by Bou et al. [16], the reduced expression of 2 porins and the presence of an OXA-derived β -lactamase were responsible for the carbapenem resistance of the epidemic nosocomial imipenem-resistant *A. baumannii* isolates. Similarly, Limansky et al. [41] have shown that the loss of a heat-modifiable 29-kDa OMP, designated CarO, was responsible for imipenem resistance (the 2 genetically related strains studied were Ab288 [imipenem susceptible] and Ab242 [imipenem resistant]) and that loss of this OMP could be readily obtained by serial passage.

Aminoglycoside-modifying enzymes (AMEs). Resistance to aminoglycosides by AMEs is also a major unwelcome feature in the multidrug-resistant phenotype of *A. baumannii* [18]. All 3 types of AMEs—the acetylating, adenylating, and phosphorylating AMEs—have been identified in *A. baumannii* [18, 42, 43]. Contemporary genetic analyses have been devoted to investigating the nature of these AMEs, since many of them have been encoded on integrans.

Quinolone resistance. Molecular analysis of a collection of quinolone-resistant *A. baumannii* isolates by Vila et al. [44, 45] revealed that mutations in both in *gyrA* and *parC* are responsible for quinolone resistance. The plasmid-mediated quinolone resistance gene, *qnrA*, has not yet been detected in *A. baumannii*, although it has been found in other gram-negative bacteria, such as *Enterobacter* and *Klebsiella* species [46]. The Qnr protein protects DNA from quinolone binding [47].

Efflux pump. The natural role of efflux is to remove chem-

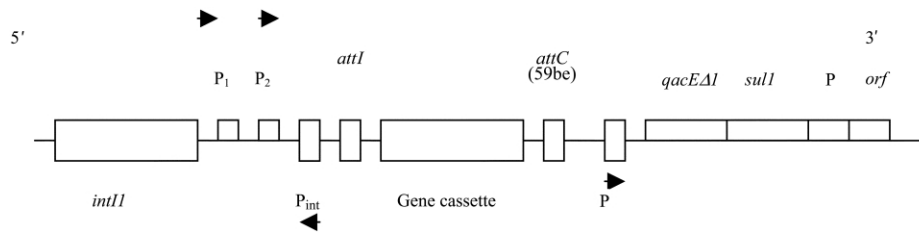


Figure 1. Simplified representation of a class 1 integron. *attI*, integration site; *attC*, sequence in the gene cassette recognized by the integrase; P, promoter; P₁, promoter for the gene cassette; P₂, second promoter; P_{int}, promoter for the integrase; *orf*, open reading frame; *qacEΔ1*, partially deleted gene that encodes resistance to a quaternary ammonium compound; *sull*, gene for sulfonamide resistance. As pictured, integrons contain components of a site-specific recombination system that recognizes and captures mobile gene cassettes. Gene cassettes can be antibiotic resistance genes followed by a repeat sequence called a 59-bp element (59be) or *attC*. In *Acinetobacter baumannii*, gene cassettes may contain β-lactamase genes (e.g., *bla*_{IMP-4}, *bla*_{VIM-1}, and *bla*_{OXA}).

icals that could potentially disorganize the cytoplasmic membrane; however, from the point of view of antibiotic resistance, efflux pumps have a potent ability to actively expel β-lactams, quinolones, and sometimes even aminoglycosides. Efflux pumps usually have 3 components: the pump itself, which lies in the cytoplasmic membrane; an exit portal (porin channels traversing the outer membrane); and a linker lipoprotein between the two. An RND-type efflux pump has been described in *A. baumannii* [48, 49]. By inactivation of *adeB*, it was shown that this efflux pump was responsible for aminoglycoside resistance and was involved in resistance to quinolones, tetracyclines, chloramphenicol, erythromycin, trimethoprim, and ethidium bromide. Marchand et al. [49] showed that the AdeABC pump is cryptic in wild-type *A. baumannii* because of stringent control by a 2-component regulatory and sensor system.

ANTIBIOTIC RESISTANCE IN *P. AERUGINOSA*

AmpC cephalosporinase. AmpC cephalosporinase is characteristically chromosomally encoded in *P. aeruginosa*. Some antibiotics, such as the carbapenems, are strong inducers of this β-lactamase but are, fortunately, stable to its hydrolytic effects. Interestingly, clavulanate can induce expression of the AmpC β-lactamase, resulting in antagonism of the bactericidal activity of ticarcillin [50, 51]. This has led some authors to suggest that ticarcillin-clavulanate be avoided when selecting an antipseudomonal β-lactam antibiotic [50]. Tazobactam does not induce hyperproduction of the AmpC β-lactamase [50].

Most importantly, antibiotic therapy selects derepressed mutants that permanently hyperproduce AmpC β-lactamase. Stably derepressed mutants that hyperproduce the AmpC β-lactamase may lead to resistance to ticarcillin, piperacillin, and third-generation cephalosporins [52].

PSE β-lactamases. *P. aeruginosa* has the ability to acquire a wide range of β-lactamases. Four PSE enzymes have been reported in *P. aeruginosa*: PSE-1 (CARB-2), PSE-4 (CARB-1), CARB-3, and CARB-4 [53–56]. The PSE-1 and PSE-4 enzymes are active against penicillins and are inhibited by currently avail-

able β-lactamase inhibitors but do not inactivate the antipseudomonal cephalosporins, carbapenems, or aztreonam.

OXA β-lactamases. Although OXA β-lactamases have also been reported in other gram-negative isolates, they occur predominantly in *P. aeruginosa* and *Acinetobacter* species (see above) [25, 33, 34, 57–71]. More recently, OXA-type β-lactamases with broader activity than earlier enzymes have been described. In many ways, the newer OXA-type β-lactamases have similarities with the extended-spectrum β-lactamases (ESBLs), which typically have minor sequence substitutions compared with the genes of parent β-lactamases. These sequence changes greatly increase the spectrum of activity of the β-lactamases against antibiotics such as ceftazidime or aztreonam. OXA-10 mutants have ESBL activity and can hydrolyze third-generation cephalosporins and aztreonam [64]. Some mutants can hydrolyze cefepime to a much greater extent than they do ceftazidime; OXA-31 is one such example [70].

TEM-, SHV-, and other class A-type β-lactamases. TEM- and SHV-type β-lactamases have been described in *P. aeruginosa*, but very rarely [72–77]. To our knowledge, the CTX-M type ESBLs, which are now emerging as a dominant ESBL type, have never been described in *P. aeruginosa* [78].

PER-1 has been found to occur in *P. aeruginosa* [79, 80] and has been found in ~10% of nosocomial *P. aeruginosa* isolates in Turkish hospitals [20]. The presence of PER-1 β-lactamase-producing *P. aeruginosa* has also been reported from countries other than Turkey [81, 82], and nosocomial outbreaks of infection with multidrug-resistant strains producing PER-1 have also been described [83, 84]. A recent report describes poor therapeutic outcomes associated with the expression of PER-1 in *P. aeruginosa* [85]. PER-1 shows a broad substrate profile, in that it hydrolyzes benzylpenicillin, amoxicillin, ticarcillin, cephalothin, cefoperazone, cefuroxime, ceftriaxone, ceftazidime, and (moderately) aztreonam but not oxacillin, imipenem, or cephamycins.

Other less common ESBLs, such as those of VEB, GES, and IBC type, have been detected in *P. aeruginosa* isolates. VEB-1

was identified in France, in a patient who had likely imported the strain from Thailand [86]. Similar strains were recently reported from Thailand and Kuwait [77, 87, 88]. GES-1, previously described in an integron from *Klebsiella pneumoniae*, has recently been described in *P. aeruginosa* [89]. GES-2 is a point-mutant derivative of the ESBL GES-1. GES-2 was identified from a *P. aeruginosa* isolate in South Africa and later from 8 more strains involved in the outbreak [90, 91]. An ESBL, IBC-2, produced by a clinical strain of *P. aeruginosa*, was found, as a gene cassette, to be the sole gene within the variable region of a class 1 integron probably located in the chromosome. IBC-2 is a variant of IBC-1 (originally found in *Enterobacter cloacae*) and GES-1 (originally found in *K. pneumoniae*), differing by 1 aa from each of these β -lactamases [92]. These ESBLs share the ability to render inactive the third-generation cephalosporins, penicillins, and aztreonam. In the vast majority of situations, they do not affect the carbapenems. However, GES-2 can hydrolyze imipenem (but not meropenem) [90].

Metallo-carbapenemases. In general, carbapenem resistance in *P. aeruginosa* attributed to β -lactamases is due to metallo- β -lactamases. The major types that have been identified are IMP, VIM, SPM, and GIM [93, 94]. The production of these metallo- β -lactamases by *P. aeruginosa* can lead to resistance to imipenem and meropenem plus the antipseudomonal cephalosporins, including cefepime, and antipseudomonal penicillins [93]. These β -lactamases do not destroy aztreonam. These carbapenemases are not inhibited by clavulanic acid, tazobactam, or sulbactam; hence, addition of tazobactam to piperacillin or clavulanic acid to ticarcillin does not add to the activity of these penicillins against metallo- β -lactamase-producing strains.

The IMP and VIM β -lactamases share <40% amino acid identity but exhibit comparable kinetic properties, inactivating virtually all β -lactams except monobactams [93]. Additionally, both *bla*_{IMP}- and *bla*_{VIM}-type genes are carried as gene cassettes by class 1 integrons. SPM is a distinctly different metallo- β -lactamase from VIM and IMP and, accordingly, represents a new subfamily of mobile metallo- β -lactamases. However, SPM-1 appears to preferentially hydrolyze cephalosporins, although the hydrolytic profile of SPM-1 bears the most similarity to that of IMP-1 [95].

IMP-1 was first reported in *Serratia marcescens* and *P. aeruginosa* isolates [96–98]. The IMP-type β -lactamases have a truly global distribution [99–101].

Even though VIM enzymes have <40% amino acid homology with the IMP enzymes, they have the same antibiotic spectrum profile [93]. VIM-1 was the first to be identified in *P. aeruginosa* [102]. Numerous outbreaks of infection with VIM-2 have since been described [84, 103–114]. Most recently, a nosocomial outbreak of infection with VIM-2-producing *P. aeruginosa* occurred in Chicago [115].

OMP (porin) changes. Despite the occurrence of carbapenemases, the most common means by which *P. aeruginosa* isolates become imipenem resistant is via mutational loss of a 54-kDa OMP [116]. This protein is usually known as OprD (or the D2 porin). Loss of OprD production is likely due to inactivation of the OprD gene [117]. Loss of OprD causes imipenem resistance; isolates that have lost OprD will have reduced susceptibility to meropenem, although this does not usually lead to resistance as defined by conventional break points. Loss of OprD does not confer resistance to β -lactams other than the carbapenems. Mutational loss of OprD is frequent during imipenem therapy: in a variety of clinical studies, imipenem resistance has emerged during treatment of *P. aeruginosa* infections in ~25% of patients treated with that drug [118–121].

AMEs. An increasing complexity of aminoglycoside resistance mechanisms is being observed in *P. aeruginosa*, including impermeability, multidrug active efflux systems, and enzymatic modification of the amino or hydroxyl groups of the aminoglycosides [122]. As in *Acinetobacter* species, AMEs are common in *P. aeruginosa*, especially AAC(6)-I and APH(3)-II [123, 124], but are not the sole mechanism of aminoglycoside resistance. Novel aminoglycoside resistance gene cassettes are being discovered; in the worst-case scenario, these are being discovered within integrons that also encode metallo- β -lactamases [86].

Quinolone resistance. As in *Acinetobacter* species, quinolone resistance may be attributable to mutation in the regulator genes for the efflux system or to mutations of the target enzymes—topoisomerases II and IV (encoded by *gyrA* and *parC*, respectively). However, it seems that mutations in the genes encoding the topoisomerases (especially *gyrA*) are the most important. Both high- and low-level ciprofloxacin resistance are associated with a mutation in *gyrA*. Mutations in *parC* are found in highly resistant isolates when joined with mutations in *gyrA*. Mutations in the efflux regulatory genes are associated with high-level resistance only when they appear together with a mutation in *gyrA* or *parC* [125].

Efflux pumps. Efflux pump systems are emerging as extremely important causes of multidrug resistance in *P. aeruginosa* [126]. The terminology of the commonly observed efflux pump system in *P. aeruginosa* is logical, in that the name is a compound of the designations for the pump, the linker lipoprotein, and the exit portal. For example, the most commonly observed pump system, MexAB-OprM, comprises a pump (MexB), a linker lipoprotein (MexA), and an exit portal (OprM).

The MexAB-OprM system, when up-regulated, leads to resistance to the quinolones, the antipseudomonal penicillins, and the antipseudomonal cephalosporins. Meropenem susceptibility may decrease, but imipenem susceptibility is usually not affected. This is a key discriminating factor. Aminoglycoside susceptibility is not affected by this pump [126]. In contrast, up-regulation of the MexXY-OprM efflux pump system does

affect aminoglycoside susceptibility. The MexXY-OprN efflux pump system is unusual, in that it is coregulated with OprD. Mutants with up-regulated MexXY-OprN and with reduced OprD will be resistant to multiple drugs, including both imipenem and meropenem, quinolones, antipseudomonal penicillins, aztreonam, and antipseudomonal cephalosporins [127].

Membrane changes and resistance to polymyxins. Resistance to colistin in *P. aeruginosa* (and *A. baumannii*) is rare but has been found [100]. Structural modifications of the outer cell membrane are thought to be responsible for high-level resistance of *P. aeruginosa* to colistin [128]. Such changes include the absence of 2-hydroxylaurate, the presence of 4-aminoarabinose, and an increase in the palmitate content of lipid A. More research is needed to adequately characterize mechanisms of polymyxin resistance.

Panresistance. Resistance to all antibiotics except the polymyxins is now a reality in many medical centers. Panresistance typically is the result of the convergence of multiple resistance mechanisms. Deplano et al. [129] described an outbreak of panresistant *P. aeruginosa* in an intensive care unit in Belgium. The isolates overexpressed the chromosomal β -lactamase AmpC and had decreased expression of the porin OprD. Additionally, up-regulation of the MexXY efflux system was present. Lolans et al. [115] described an outbreak of panresistance in *P. aeruginosa* in an intensive care unit in Chicago. The isolates produced a metallo- β -lactamase enzyme (VIM-2), the chromosomally encoded AmpC β -lactamase, and the genes encoding 2 AMEs (*aacA7* and *aacC-A5*). The isolates were variably resistant to aztreonam—metallo-enzymes do not hydrolyze aztreonam. However, the coexistence of other resistance mechanisms is likely to compromise susceptibility to aztreonam.

WHY RESISTANCE TO SO MANY ANTIBIOTICS?

Perhaps the intrinsic impermeability of their outer membranes coupled with the close relationship of *A. baumannii* and *P. aeruginosa* to the soil and aquatic environment has made it possible for these organisms to acquire highly effective resistance determinants in response to multiple challenges. In a study by D'Costa et al. [130], the soil was demonstrated to be a reservoir of resistance genes (the “antibiotic resistome”). In *Acinetobacter calcoaceticus*, the permeability coefficients of zwitterionic cephalosporins were 2–7 times lower than the permeability coefficients of the same β -lactams in the outer membrane of *P. aeruginosa* [131]. The diffusion rates of carbapenems and zwitterionic cephalosporins into liposomes containing purified outer membrane appeared to be ~1%–3%, that of the *E. coli* outer membrane. It remains to be established whether the same mechanism is operating in *A. baumannii*. We believe that the ability of this pathogen to harbor diverse genetic elements parallels the experience with *P. aeruginosa*. Genome-wide analysis will provide critical insights into this ability.

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