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Multiple Mechanisms of Antimicrobial Resistance in *Pseudomonas aeruginosa:* Our Worst Nightmare?

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Pseudomonas aeruginosa **carries multiresistance plasmids less often than does** ***Klebsiella pneumoniae,*** **develops mutational resistance to cephalosporins less readily than** ***Enterobacter*** **species, and has less inherent resistance than** ***Stenotrophomonas maltophilia.*** **What nevertheless makes** ***P. aeruginosa*** **uniquely problematic is a combination of the following: the species' inherent resistance to many drug classes; its ability to acquire resistance, via mutations, to all relevant treatments; its high and increasing rates of resistance locally; and its frequent role in serious infections. A few isolates of** ***P. aeruginosa*** **are resistant to all reliable antibiotics, and this problem seems likely to grow with the emergence of integrins that carry gene cassettes encoding both carbapenemases and amikacin acetyltransferases.**

Even drug-susceptible strains of *Pseudomonas aeruginosa* have considerable defenses. Like some Enterobacteriaceae species, *P. aeruginosa* has an inducible AmpC β-lactamase and is inherently resistant to those β-lactams that induce this enzyme and are hydrolyzed by it (e.g., cephalothin and ampicillin) [1]. Moreover, many antibiotics are excluded from the pseudomonal cell. This exclusion long was attributed to the cell's impermeability, although evidence of this was scanty and although the belief proved difficult to reconcile with the discovery that *P. aeruginosa* copiously manufactures a porin (OprF) that forms large outer membrane pores [2]. In the early 1990s, it began to be realized that much of this "impermeability-mediated resistance" (as it was widely called at the time) actually reflected efflux by MexAB-OprM (figure 1), a pump system that removes β-lactams, chloramphenicol, fluoroquinolones, macrolides, novobiocin, sulfonamides, tetracycline, and trimethoprim, as well as various dyes and detergents [3]. The natural role of MexAB-OprM may be to remove amphipathic permeants, which otherwise would disorganize the cytoplasmic membrane.

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Although efflux is the greater factor, the role of impermeability in pseudomonal resistance was confirmed recently by Li et al. [4], who showed that membrane disorganizers, such as EDTA, potentiated antibiotics, especially in the absence of MexAB-OprM. The MICs for a highly permeable and MexAB-OprM–deficient mutant were as follows: carbenicillin, 0.0125 mg/L; ciprofloxacin, 0.03 mg/L; tetracycline, 0.015 mg/L; and chloramphenicol, 0.5 mg/L. These MIC values are considerably less than those for clinical isolates. This impermeability can be reconciled with copious production of OprF if many pores are nonfunctional or if they adopt a smaller diameter in the membrane than in reconstituted liposomes.

MUTATIONS AND RESISTANCE

Various penicillins, cephalosporins, carbapenems, monobactams, aminoglycosides, fluoroquinolones, and polymyxins overcome the inherent defenses of *P. aeruginosa* and are active against most isolates. All, however, are prone to being compromised by mutational resistance (table 1). Mutations to topoisomerases II and IV confer fluoroquinolone resistance more readily in *P. aeruginosa* than in Enterobacteriaceae, because *P. aeruginosa* has poorer inherent susceptibility [5]. Derepression of the chromosomal AmpC β-lactamase reduces susceptibility to penicillins and cephalosporins, although the level of resistance depends on the degree of derepression, which is more variable than that in *Enterobacter* mutants [1]. The up-regulation of MexAB-OprM compromises

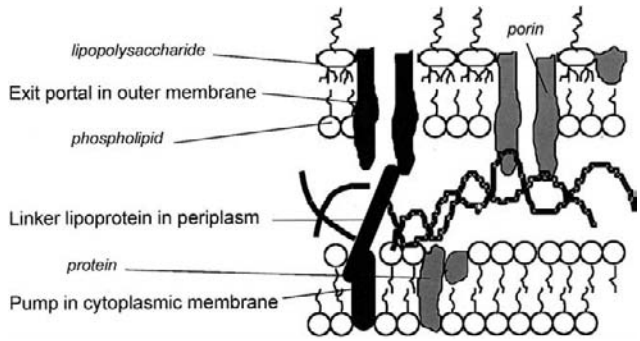

Figure 1. Three-component efflux pump. The pump itself (MexB, MexD, or MexF, according to the system) lies in the cytoplasmic membrane and is attached via a linker lipoprotein (MexA, MexC, or MexE) to the exit portal (OprM, OprJ, or OprN). Efflux system components appear in large roman type; other membrane components appear in small italic type.

the fluoroquinolones, penicillins, cephalosporins, and, to some extent, meropenem (although not imipenem), and it also enhances resistance to many other drugs that lack useful antipseudomonal activity [3, 6]. Up-regulation of other efflux systems—for example, MexCD-OprJ and MexEF-OprN—confers resistance to fluoroquinolones and some β-lactams; up-regulation of MexXY-OprM also affects aminoglycosides. Genome sequencing suggests that there are at least 5 additional efflux systems, all of which await characterization [3].

Permeability mutations are widely blamed for increased resistance to β-lactams and fluoroquinolones, but, again, much of what was once attributed to impermeability is now understood to reflect up-regulated efflux. Reduced expression of OprF has only a slight effect on the MICs of β-lactams and fluoroquinolones [7]. There is better evidence that increased impermeability is a mechanism of aminoglycoside resistance—for example, in the "small-colony variants" that are sometimes selected during gentamicin therapy and in isolates with reduced susceptibility to all aminoglycosides [8]. Because these latter organisms lack modifying enzymes and are not cross-resistant to fluoroquinolones or β-lactams, impermeability is more likely to be the mechanism of resistance than is up-regulation of MexXY-OprM, which affects other antibiotic classes in addition to aminoglycosides (table 1).

Mutational impermeability is important in resistance to carbapenems and arises via the loss of OprD, a porin that forms narrow transmembrane channels that are accessible to carbapenems but not to other β-lactams [9]. Loss of OprD is associated with resistance to imipenem and reduced susceptibility to meropenem. To complicate matters, OprD is coregulated with MexEF-OprN; thus, the *nfxc (mexT)* mutants that occasionally are selected by fluoroquinolones (not carbapenems) have (1) up-regulated MexEF-OprN and reduced OprD, with consequent resistance to both fluoroquinolones and imipenem, and (2) reduced susceptibility to meropenem [10]. Polymyxins were long thought to evade stable mutational resistance, but MICs of 128–256 mg/L have been noted for a few isolates from patients with cystic fibrosis, who receive the nebulized drug for

Table 1. Mutational resistances in *Pseudomonas aeruginosa.*

		Effect on strain, according to antipseudomonal drug								
Mechanism	Mutation site	Fq	Carb-Tic	Pip-Azl	Czid-Atm	Cpm-Cpr	Imi	Mero	Agl	Pm
Reduced affinity										
Of topoisomerase II	*gyrA*	r/R	—	—	—	—	—	—	—	—
Of topoisomerase IV	*parC*	r/R	—	—	—	—	—	—	—	—
Derepression of AmpC										
Partial	*ampD*	—	R	R	R	r	—	—	—	—
Total	*ampD* + other	—	R	R	R	R	—	—	—	—
Up-regulation										
Of MexAB-OprM	*nalB* at *mexR*; *nalC* at other	R/R	R	r/R	r/R	r/R	—	r	—	—
Of MexCD-OprJ	*nfxB*	r/R	r/R	r/R	r/R	R	—	r	—	—
Of MexEF-OprN	*nfxC* at *mexT*	r/R	r/R	r/R	r/R	r/R	r	r	—	—
Of MexXY-OprM		r/R	r/R	r/R	r/R	r/R	—	r	r/R	—
Reduced aminoglycoside transport		—	—	—	—	—	—	—	r/R	—
Loss of OprD	*oprD*; *nfxC* at *mexT*	—	—	—	—	—	R	r	—	—
Membrane changes		—	—	—	—	—	—	—	—	R

NOTE. Agl, aminoglycosides; Azl, azlocillin; Atm, aztreonam; Carb, carbenicillin, Czid, ceftazidime, Cpm, cefepime; Cpr, cefpirome; Fq, fluoroquinolone; Imi, imipenem; Mero, meropenem; Pip, piperacillin; Pm, polymyxin; r, reduced susceptibility; R, frank resistance, which may vary in its distinction from "r," according to the breakpoints adopted; Tic, ticarcillin.

Table 2. Extended-spectrum metallo–β-lactamases found in *Pseudomonas aeruginosa.*

Enzyme(s)	Country where strain was found	Encodement site	Associated phenotype, by drug						Inhibition by	
			Carb-Tic	Pip-Azl	Czid	Cpm-Cpr	Atm	Imi-Mero	Clv	Taz
PER-1	Turkey (mostly), Italy, France, Belgium	Plasmids or chromosome	R	r	R	R	R	S	Strong	Weak
OXA-11, -14, -16, -19, -28	Turkey (OXA-11, -14, and -16); France (OXA-19 and -28)	Integrins in plasmids or chromosome	R	R	R	R	R	S	Weak	Weak
OXA-15	Turkey	Plasmids	R	R	R	R	R	S	Weak	Weak
IMP-1/-8	Japan (IMP-1); Canada (IMP-7)	Integrins in plasmids or chromosome	R	R	R	R	S	r/R	No	No
VIM types	Italy (VIM-1), France, Greece, Korea (VIM-2), Taiwan (VIM-8)	Integrins in plasmids or chromosome	R	R	R	R	S	r/R	No	No

NOTE. Azl, azlocillin; Carb, carbenicillin, Clv, clavulanate; Cpm, cefepime; Cpr, cefpirome; Imi, imipenem; Czid, ceftazidime; Mero, meropenem; Pip, piperacillin; r, reduced susceptibility; R, frank resistance, which may vary in its distinction from "r," according to the breakpoints adopted, the permeability of the strain, and the amount of enzyme produced; S, susceptible; Taz, tazobactam; Tic, ticarcillin.

long periods. The mechanisms at work are unclear, but they probably involve changes to membrane architecture.

MULTIPLE MUTATIONS AND MULTIDRUG RESISTANCE

No single mutation compromises every antipseudomonal drug. Nevertheless, up-regulated efflux can simultaneously compromise fluoroquinolones and most β-lactams, leaving only the aminoglycosides (which lack reliable efficacy as antipseudomonal monotherapy) and imipenem (to which mutational resistance evolves at high frequency). A combination of up-regulated efflux, loss of OprD, and impermeability to aminoglycosides compromises every drug class except the polymyxins. Each of the necessary mutations arises in 1 cell per 10^7 to 10^9 cells, and, although simultaneous emergence is mathematically and biologically improbable, sequential emergence is all too likely because infections that are resistant to the first antibiotic administered are likely to be treated with a second antibiotic, and so on. Mutations that up-regulate efflux may act additively with those that effect permeability, β-lactamase expression, or topoisomerase susceptibility so as to exacerbate resistance [11].

Accumulation of sequential mutations may be facilitated by hypermutators, which either lack the ability to perform DNA proofreading or mismatch repair, or which use DNA polymerases with a reduced copying fidelity. Because resistance is most likely to emerge in hypermutators, antibiotics may select for hypermutators, thereby increasing the probability that further resistance will emerge. Hypermutators were found in sputum samples obtained from 11 of 30 patients who had cystic fibrosis and chronic *P. aeruginosa* infections, compared with 0 of 75 samples from patients without cystic fibrosis who had acute infections [12].

ACQUIRED GENES AND MULTIDRUG RESISTANCE

Many acquired β-lactamases and aminoglycoside-modifying enzymes have been noted in *P. aeruginosa.* Some of these are widely prevalent among isolates from southern Europe, Turkey, and Southeast Asia, although they are not widely prevalent in the United Kingdom. The most frequently acquired β-lactamases are PSE-1 and PSE-4. Like classical TEM and OXA enzymes (which also occur, albeit rarely, in *P. aeruginosa*), these PSE enzymes can be circumvented with the use of carbapenems, oxyimino-aminothiazolyl cephalosporins (e.g., ceftazidime, cefepime, or cefpirome) or with monobactams [1]. However, β-lactamases that give wider resistance are emerging in *P. aeruginosa.* PER-1 β-lactamase and the extended-spectrum OXA types (known as OXA–extended-spectrum β-lactams, or OXA-ESBLs) deserve discussion, as do the IMP and VIM metallo–β-lactamases (table 2).

PER-1, a class A β-lactamase, confers high-level resistance to ceftazidime, with susceptibility restored by the addition of clavulanate, but it has little in vitro effect on piperacillin; carbapenems are stable. PER-1 is frequently noted in *P. aeruginosa* from Turkey [13], and it occasionally has been found in Europe. OXA-ESBLs, like PER-1, mainly are reported in *P. aeruginosa* from Turkey and include OXA-11, -14, -16, -17, -19, and -28 mutants of OXA-10, as well as the OXA-15 mutant of OXA-2 [14, 15]. Like TEM and SHV ESBLs, the OXA ESBLs have minor sequence substitutions that greatly extend their hydrolytic spectra. All give resistance to oxyimino-aminothiazolyl cephalosporins, monobactams, and penicillins, but not to carbapenems. A few *P. aeruginosa* isolates from Turkey produce both PER enzymes and ESBLs, often together with potent aminoglycoside-modifying enzymes [16].

IMP and VIM are metallo–β-lactamases that rapidly hydrolyze penicillins, cephalosporins, and carbapenems—but not az-

Table 3. Trends in resistance among *Pseudomonas aeruginosa* isolates in the United States and the United Kingdom.

Site (year) and drug	Breakpoint,[a] mg/L		Percent resistance, by patient isolate				
	Susceptible	Resistant	All	Inpatient	ICU	Outpatient	CF sputum
United States (2000)							
Ceftazidime	≤8	≥32	11.5	13.2	18.2	8.3	18.8
Piperacillin	≤64	≥128	15.8	17.9	23.5	12.0	19.8
Imipenem	≤4	≥16	14.2	15.5	22.5	11.4	14.2
Amikacin	≤16	≥64	7.1	6.1	6.0	8.8	26.4
Gentamicin	≤4	≥16	19.1	18.9	22.3	18.7	41.1
Ciprofloxacin	≤1	≥4	29.5	31.2	32.2	27.0	23.3
United Kingdom (1999)							
Ceftazidime	≤8	≥16	2.3	2.3	2.9	1.0	14.0
Piperacillin	≤16	≥32	3.9	4.3	4.6	2.3	11.0
Imipenem	≤4	≥8	8.1	7.2	15.6	4.4	31.0
Amikacin	≤4	≥32	5.6	3.7	1.2	4.7	36.0
Gentamicin	≤1	≥8	11.1	9.4	5.2	9.6	43.0
Ciprofloxacin	≤1	≥8	8.1	8.5	6.4	6.3	24.0

NOTE. US data are from The Surveillance Network Database USA (TSN; Focus Technologies). All US data, except for the data on resistance to piperacillin among CF sputum isolates, are for >1000 isolates. More than >250 US hospitals participated; >70,000 isolates were tested, but not all were tested with every antibiotic. UK data are from Henwood et al. [22] and are based on a survey of 2194 isolates from 25 hospitals. All isolates were tested with every antibiotic, with >100 isolates per group. CF sputum, sputum isolates from patients with cystic fibrosis; ICU, intensive care unit.

[a] Several breakpoints of the British Society for Antimicrobial Chemotherapy are more conservative than those of the National Committee for Clinical Laboratory Standards, which means that comparisons between the countries require caution.

treonam [17]. Resistance to penicillins and cephalosporins usually accompanies production, but carbapenem resistance may also require the loss of OprD. Since 1988, IMP-1 has been found in Japan, where it is now widely scattered—although still rare—among *P. aeruginosa* strains [18]. It has not been found among *P. aeruginosa* strains elsewhere; however, a related enzyme, IMP-7, was noted in *P. aeruginosa* in Canada [19], whereas IMP-2, -3, -4, -5, -6, and -8 were found in other species and countries [17]. VIM-1 was found in *P. aeruginosa* in Italy. VIM-2, the amino acid sequence of which is 90% homologous to that of VIM-1, has since been found in France, Greece, and South Korea, whilst VIM-3 has been found in Taiwan. VIM enzymes resemble IMP types with regard to their hydrolytic properties, they but share only 30%–40% amino acid identity [17].

The genes for VIM and IMP enzymes, like those for OXA-ESBLs, are often carried as cassettes within integrins, which are natural recombination systems that assemble series of acquired genes behind a single promoter. This organization facilitates gene recombination. Critically, the β-lactamase genes are often adjacent to aminoglycoside 6′-*N* acetyltransferase [*aac(6′)-1b*] determinants [17, 20, 21]. A *P. aeruginosa* strain with this combination of cassettes is susceptible only to polymyxins, ciprofloxacin, and, perhaps, aztreonam. If MexAB-OprM is up-reg-

Table 4. Resistance and multidrug resistance among *Pseudomonas aeruginosa* isolates tested with a panel of 6 antibiotics in the United States, 1997–2000.

Year	No. of isolates tested[a]	No. (%) of isolates resistant to							
		0 drugs	1 drug	2 drugs	3 drugs	4 drugs	5 drugs	6 drugs	≥3 drugs
1997	8430	5002 (59.3)	1466 (17.4)	881 (10.5)	518 (6.1)	329 (3.9)	157 (1.9)	77 (0.9)	1081 (12.8)
1998	9422	5411 (57.4)	1636 (17.4)	1019 (10.8)	623 (6.6)	428 (4.5)	232 (2.5)	73 (0.8)	1356 (14.4)
1999	11,522	5991 (52.0)	2194 (19.0)	1430 (12.4)	867 (7.5)	617 (5.4)	341 (3.0)	82 (0.7)	1907 (16.6)
2000	8016	4000 (49.9)	1360 (17.0)	988 (12.3)	661 (8.2)	514 (6.4)	414 (5.2)	79 (1.0)	1668 (20.8)

[a] Only isolates tested with the 6 drugs amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, and piperacillin (or ticarcillin) were considered in this analysis. The isolates were from >250 hospitals participating in The Surveillance Network Database USA (TSN [Focus Technologies]; data presented with permission).

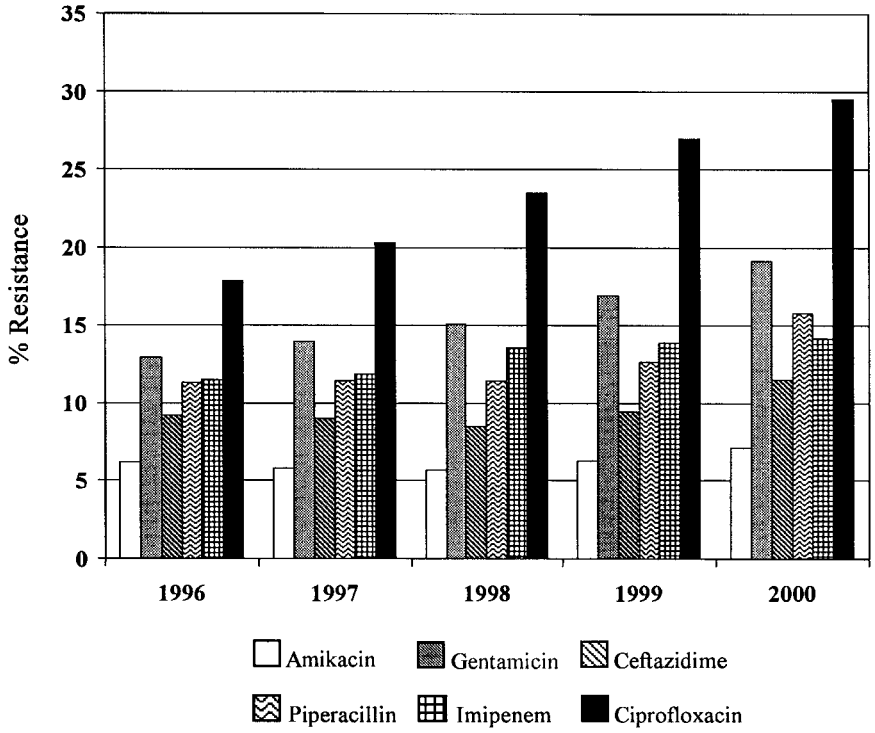

Figure 2. Percent resistance among *Pseudomonas aeruginosa* strains in the United States, 1996–2000. Data are from The Surveillance Network Database USA (TSN; Focus Technologies). Depending on the year, 58–258 hospitals participated and 24,000–70,000 isolates were tested; however, not all isolates underwent testing with all antibiotics.

ulated by mutation, or if the isolate has topoisomerase and *ampD* mutations, then only susceptibility to polymyxins remains.

PREVALENCE OF RESISTANCE AND MULTIDRUG RESISTANCE

Relevant β-lactams and aminoglycosides remain active against 70%–98% of *P. aeruginosa* isolates in the United States and the United Kingdom (tables 3 and 4). Nevertheless, resistance is more frequent locally—most notably, in units for the management of patients with burns or cystic fibrosis or in intensive care units. The excess of aminoglycoside resistance in isolates from patients with cystic fibrosis is striking in both the United Kingdom and the United States (table 3), as is the excess of imipenem resistance among isolates from patients in intensive care units. More generally, there is an excess of resistance among isolates from hospitalized patients, compared with those from outpatients. Snapshot surveys performed in 1982, 1993, and 1999 show little increase in resistance among *P. aeruginosa* strains in the United Kingdom [22]; however, resistance—to ciprofloxacin in particular but, also, to piperacillin and gentamicin—has recently risen among such strains in the United States (figure 2).

Much resistance is concentrated in a few strains. Itokazu et al. [23] compared ceftazidime-resistant (14.2%) and ceftazidime-susceptible (85.8%) organisms among a total of 6675 *P. aeruginosa* isolates collected from patients in intensive care units across the United States from 1990 to 1993. Isolates that were resistant to ceftazidime had higher rates of resistance to gentamicin (54.5% resistance to gentamcin vs. 31.7% among those susceptible to to ceftazidime), amikacin (26.9% vs. 7.8%), and imipenem (26.4% vs. 10.1%), despite the fact that resistance to aminoglycosides and imipenem is mechanistically independent of most ceftazidime resistance (table 1). The *P. aeruginosa* data collected by The Surveillance Network Database USA (Focus Technologies; Herndon, VA) likewise show rising proportions of isolates that are resistant to multiple antibiotics, with ~16% of isolates now resistant to $\geqslant$3 of the core drugs (amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, and piperacillin-ticarcillin) and with 1% resistant to all 6 of these agents (table 4). It seems likely that most of this multidrug resistance reflects the accumulation of multiple mutations, although this surmise remains to be confirmed by molecular studies, and although reports from other parts of the world document extreme multidrug resistance associated with acquired resistance genes.

In a hospital in Thessaloniki, Greece, a serotype O:12 strain

with a VIM β-lactamase and cross-resistance to aztreonam, aminoglycosides, and ciprofloxacin persisted for 3 years, with >211 isolates of this strain recovered [24]. In South Korea, VIM-2 producers are widespread in *P. aeurginosa,* with the enzyme being found in organisms at 9 of 29 hospitals surveyed (K. Lee, J. B. Lim, J. H. Yum, D. Yong, Y. Chong, J. M. Kim, and D.M.L., personal communication). Moreover, detailed study at one Korean hospital revealed dissemination of bla_{VIM}, linked to *aac6′-1b,* in multiple *P. aeruginosa* lineages. Some isolates remained susceptible to aztreonam, but others did not.

PREVENTING AND MANAGING MULTIDRUG RESISTANCE

The selection of resistant mutants, a risk associated with any antipseudomonal therapy, varies with the type and dosage of antibiotic used and the infection site. Carmeli et al. [25] found a 2-fold greater risk of selection for resistance when imipenem, rather than ciprofloxacin, ceftazidime, or piperacillin, was used; however, translation of this observation into risk management is complicated by the fact that the mechanism of resistance to imipenem is narrow in spectrum and, arguably, is less disturbing than the broader-spectrum mechanisms of resistance selected by other antibiotics. Against drug-susceptible *P. aeruginosa,* there is a strong argument for the use of meropenem rather than other β-lactams, because development of frank resistance to meropenem requires 2 mutations (up-regulated efflux and loss of OprD), whereas resistance to other drugs can arise as a result of single mutations. The differences in pseudomonal behavior in response to meropenem and imipenem have been discussed extensively elsewhere [26]. It is often assumed that combination therapy will prevent the selection of mutational resistance, but evidence for this is scanty. In addition, single efflux mutations may affect both the β-lactams and the fluoroquinolones, thereby undermining the use of combinations of these drugs (table 1).

The original emergence of multidrug resistance in association with plasmids and integrins is less predictable than mutational resistance because it depends on the random escape of genes to mobile DNA. However, once such resistance has emerged, either the host strain can spread among patients or the resistance can disseminate among strains. The former situation occurs more frequently, as with the dissemination of *P. aeruginosa* of serotype O:12 with the VIM-2 enzyme in Thessaloniki, Greece [24]. In such cases, the best answers to preventing dissemination of resistance are infection control and elimination of the unnecessary use of broad-spectrum antibiotics. In a few cases—for example, in outbreaks involving VIM-2 in Korea, IMP-1 in Japan, and PER-1 in Turkey—plasmids coding potent β-lactamases together with aminoglycoside-modifying enzymes have disseminated among *P. aeruginosa* strains, rendering control even more difficult.

When strains have multiple mutational or acquired resistances, the choice of therapy is often frighteningly limited, especially because most clinicians would prefer to use a synergistic combination for serious pseudomonal infections. No new fluoroquinolone offers better antipseudomonal activity than does ciprofloxacin, and none retains activity against ciprofloxacin-resistant isolates. Where resistance is mutational, tobramycin and meropenem are the drugs most likely to retain activity, because they are the aminoglycoside and the β-lactam with the best inherent activity against *P. aeruginosa.* Isolates with efflux-mediated resistance to meropenem, penicillins, and cephalosporins might, however, retain susceptibility to imipenem, and, although meropenem is usually the more active carbapenem, this possibility should always be considered. Carbapenems retain activity against isolates with PER enzymes and ESBLs, and monobactams retain activity against some isolates with IMP or VIM enzymes. Isepamicin (an aminoglycoside that is often available for compassionate use, even in areas where it is not licensed for such use) evades the aminoglycoside-modifying AAC(6′) enzymes that often accompany potent β-lactamases and might be worth considering.

In instances in which all chances of β-lactam, aminoglycoside, and quinolone use are lost, the polymyxins remain drugs of last resort; despite their significant toxicity, they have been used with some success. Levin et al. [27] reported that the use of intravenous polymyxin E (colistin) was successful in 35 (58%) of 60 patients treated for multidrug-resistant *Pseudomonas* and *Acinetobacter* infections, although it was associated with a failure rate of 75% when used for the treatment of pneumonias.

Perhaps most disturbing is the dearth of new drug options. Clinafloxacin was slightly more active than ciprofloxacin, but its development has been suspended, and no other antipseudomonal antibiotic is in advanced development. For the long term, multidrug efflux inhibitors are promising for use with fluoroquinolones or β-lactams [28], and metallo–β-lactamase inhibitors [29] are the focus of laboratory investigation. Unless new drugs are developed, it is hard to escape the conclusion that multidrug-resistant *Pseudomonas* strains will be an increasing reality and that the use of polymyxins will increase, despite their toxicity.

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References

1. Livermore DM. β-Lactamases in laboratory and clinical resistance. Clin Microbiol Rev **1995**; 8:557–84.
2. Benz R, Hancock RE. Properties of the large ion-permeable pores formed from protein F of *Pseudomonas aeruginosa* in lipid bilayer membranes. Biochim Biophys Acta **1981**; 646:298–308.
3. Poole K. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. J Mol Microbiol Biotechnol **2001**; 3:255–64.
4. Li XZ, Zhang L, Poole K. Interplay between the MexA-MexB-OprM multidrug efflux system and the outer membrane barrier in the multiple antibiotic resistance of *Pseudomonas aeruginosa.* J Antimicrob Chemother **2000**; 45:433–6.
5. Jalal S, Wretlind B. Mechanisms of quinolone resistance in clinical strains of *Pseudomonas aeruginosa.* Microb Drug Resist **1998**; 4:257–61.
6. Masuda N, Sakagawa E, Ohya S, Gotoh N, Tsujimoto H, Nishino T. Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM efflux pumps in *Pseudomonas aeruginosa.* Antimicrob Agents Chemother **2000**; 44:3322–7.
7. Woodruff WA, Hancock RE. Construction and characterization of *Pseudomonas aeruginosa* protein F–deficient mutants after in vitro and in vivo insertion mutagenesis of the cloned gene. J Bacteriol **1988**; 170: 2592–8.
8. Mayer KH. Review of epidemic aminoglycoside resistance worldwide. Am J Med **1986**; 80:56–64.
9. Studemeister AE, Quinn JP. Selective imipenem resistance in *Pseudomonas aeruginosa* associated with diminished outer membrane permeability. Antimicrob Agents Chemother **1988**; 32:1267–8.
10. Ochs MM, McCusker MP, Bains M, Hancock RE. Negative regulation of the *Pseudomonas aeruginosa* outer membrane porin OprD selective for imipenem and basic amino acids. Antimicrob Agents Chemother **1999**; 43:1085–90.
11. Lee A, Mao W, Warren MS, et al. Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. J Bacteriol **2000**; 182:3142–50.
12. Oliver A, Canton R, Campo P, Baquero F, Blazquez J. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science **2000**; 288:1251–4.
13. Vahaboglu H, Ozturk R, Aygun G, et al. Widespread detection of PER-1–type extended-spectrum β-lactamases among nosocomial *Acinetobacter* and *Pseudomonas aeruginosa* isolates in Turkey: a nationwide multicenter study. Antimicrob Agents Chemother **1997**; 41:2265–9.
14. Naas T, Nordmann P. OXA-type β-lactamases. Curr Pharm Des **1999**; 5:865–79.
15. Poirel L, Girlich D, Naas T, Nordmann P. OXA-28, an extended-spectrum variant of OXA-10 β-lactamase from *Pseudomonas aeruginosa* and its plasmid- and integrin-located gene. Antimicrob Agents Chemother **2001**; 45:447–53.
16. Danel F, Hall LM, Duke B, Gur D, Livermore DM. OXA-17, a further extended-spectrum variant of OXA-10 β-lactamase, isolated from *Pseudomonas aeruginosa.* Antimicrob Agents Chemother **1999**; 43:1362–6.
17. Livermore DM, Woodford N. Carbapenemases: a problem in waiting? Curr Opin Microbiol **2000**; 3:489–95.
18. Senda K, Arakawa Y, Nakashima K, et al. Multifocal outbreaks of metallo-β-lactamase–producing *Pseudomonas aeruginosa* resistant to broad-spectrum β-lactams, including carbapenems. Antimicrob Agents Chemother **1996**; 40:349–53.
19. Gibb AP, Tribuddharat C, Moore RC, et al. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new bla_{IMP} allele, bla_{IMP-7}. Antimicrob Agents Chemother **2002**; 46:255–8.
20. Lauretti L, Riccio ML, Mazzariol A, et al. Cloning and characterization of bla_{VIM}, a new integrin-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob Agents Chemother **1999**; 43:1584–90.
21. Arakawa Y, Murakami M, Suzuki K, et al. A novel integrin-like element carrying the metallo-β-lactamase gene bla_{IMP}. Antimicrob Agents Chemother **1995**; 39:1612–5.
22. Henwood CJ, Livermore DM, James D, Warner M, and the *Pseudomonas* Study Group. Antimicrobial susceptibility of *Pseudomonas aeruginosa:* results of a UK survey and evaluation of the British Society for Antimicrobial Chemotherapy disc susceptibility test. J Antimicrob Chemother **2001**; 47:789–99.
23. Itokazu GS, Quinn JP, Bell-Dixon C, Kahan FM, Weinstein RA. Antimicrobial resistance rates among aerobic gram-negative bacilli recovered from patients in intensive care units: evaluation of a national postmarketing surveillance program. Clin Infect Dis **1996**; 23:779–84.
24. Tsakris A, Pournaras S, Woodford N, et al. Outbreak of infections caused by *Pseudomonas aeruginosa* producing VIM-1 carbapenemase in Greece. J Clin Microbiol **2000**; 38:1290–2.
25. Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant *Pseudomonas aeruginosa:* comparison of risks associated with different antipseudomonal agents. Antimicrob Agents Chemother **1999**; 43:1379–82.
26. Livermore DM. Of *Pseudomonas,* porins, pumps and carbapenems. J Antimicrob Chemother **2001**; 47:247–50.
27. Levin AS, Barone AA, Penco J, et al. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* Clin Infect Dis **1999**; 28: 1008–11.
28. Lomovskaya O, Warren MS, Lee A, et al. Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa:* novel agents for combination therapy. Antimicrob Agents Chemother **2001**; 45:105–16.
29. Hammond GG, Huber JL, Greenlee ML, et al. Inhibition of IMP-1 metallo-β-lactamase and sensitization of IMP-1–producing bacteria by thioester derivatives. FEMS Microbiol Lett **1999**; 179:289–96.