Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and impact on treatment

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Abstract Pseudomonas aeruginosa continues to be a major cause of infections in Western society, in part because of its high intrinsic resistance to antibiotics. It has been demonstrated that this intrinsic resistance arises from the combination of unusually restricted outer-membrane permeability and secondary resistance mechanisms such as energy-dependent multidrug efflux and chromosomally encoded periplasmic β-lactamase. Given this high level of natural resistance, mutualistic resistance to most classes of antibiotics can readily arise. In this review we summarize new insights into the mechanisms of resistance, and describe therapeutic approaches that can be used in the face of this continuing resistance threat, as well as new approaches that are being developed to combat resistance. © 2000 Harcourt Publishers Ltd

INTRODUCTION

In recent years, the importance of antibiotic resistance in gram-positive bacteria, especially the ‘superbugs’ methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus sp. (VRE), has been emphasized as a premier threat in western society.1 Possibly because of this emphasis, we have recently seen the introduction into clinical trials, and gradually into the clinic, of novel chemical classes of antibiotics, including the streptogramin combination Synercid (quinupristin/dalfopristin), and the oxazolidinone linezolid. These compounds may well have a substantial impact on highly resistant gram-positive bacteria, but unfortunately have little, if any, meaningful activity against resistant gram-negative pathogens. Thus we can anticipate continuing problems with these latter organisms in the absence of any breakthrough therapies.

Pseudomonas aeruginosa is a gram-negative bacterium that continues to be a major cause of opportunistic nosocomial infections, causing around 9–10% of hospital infections.2 It is also the dominant cause of chronic lung infections contributing to the death of patients with cystic fibrosis. A major reason for its prominence as a pathogen is its high intrinsic resistance to antibiotics, such that even for the most recent antibiotics, a modest change in susceptibility can thwart their effectiveness.

Four years ago, we reviewed in detail the mechanisms of action and resistance and modes of clinical utilization of antibiotics for P. aeruginosa.2 In this review, we summarize relevant information on antibiotics, their usage and resistance in P. aeruginosa, concentrating on advances in the past 4 years. The most important recent event in Pseudomonas biology has been the complete sequencing of the genome of strain PA01 of this organism.3 This will undoubtedly aid in our future understanding of P. aeruginosa, and we will attempt to describe here some of the implications of knowledge of the complete gene complement of this organism. Overall, the genome sequence reveals in part the basis for the versatility of P. aeruginosa. It has the largest bacterial genome sequenced to date with 5570 genes and the largest proportion of regulatory genes with nearly 10% of all genes being devoted to orchestrating the biology of this organism. This is consistent with the observation that most of the clinically significant resistances observed in this bacterium involve regulatory mutations. However, we believe that over time, study of this regulatory network will reveal the reason behind the observation that there is often a disparity between in vitro (laboratory) and in vivo (clinic) antibiotic resistance, leading to treatment difficulties that are not easily explained by in vitro susceptibility tests.

INTRINSIC ANTIBIOTIC RESISTANCE

It is now well understood that in P. aeruginosa, as in other gram-negative bacteria, intrinsic resistance involves the collaboration of restricted uptake through the outer membrane, and secondary resistance mechanisms such as energy-dependent efflux and β-lactamase(s).5 Thus the importance of low outer-membrane permeability is clear because agents that break down the outer-membrane permeability barrier (e.g. cationic antimicrobial peptides6 or mutations to create large channels in the outer membrane) make cells more susceptible to antibiotics. Similarly, mutations in the major efflux systems involved in intrinsic resistance (see below) make cells more susceptible to multiple antibiotics, as do mutations preventing the induction of chromosomal β-lactamase.9,10 The current explosion of information on efflux in P. aeruginosa might lead one to conclude that this is the only important element of intrinsic resistance. However, there are really no major differences in mechanism or efficiency of intrinsic Pseudomonas efflux systems (compared, for example, to E. coli). It is the 10- to one 100-fold lower outer-membrane permeability of this bacterium that clearly distinguishes it from other gram-negative bacteria like E. coli.11 It is important to note, however, that low outer-membrane permeability is not a stand-alone resistance mechanism.12 Rather, it serves to decrease the rate of uptake of antibiotics such that at a given external antibiotic concentration, permeation into the cell is much slower and the secondary resistance mechanisms, such as antibiotic efflux or degradation, can work more effectively.

The outer membrane of gram-negative bacteria constitutes an asymmetric lipopolysaccharide: phospholipid bilayer that contains proteins termed porins which form water-filled channels as the major conduit for diffusion of hydrophilic molecules. Our current understanding of the porins of P. aeruginosa is as follows. OprF comprises the major porin for larger compounds such as tri- and tetra-saccharides and possibly antibiotics,12 and apparently forms a
The small channels are probably due to the N-terminal half forming an 8-stranded \( \beta \)-barrel with a small central water-filled channel that is too small to permit uptake of the above mentioned substrates.\(^{33}\) The molecular basis for forming a minority of large channels has not yet been clarified. In addition to OprF, it has been shown that the glucose-selective porin OprB permits uptake of sugars and saccharides,\(^{14}\) while specific porin OprD mediates uptake of basic amino acids and peptides, as well as glucanate and certain aromatic hydrocarbons,\(^{15}\) although there is no evidence that either have channels that could mediate uptake of most antibiotics. There are, however, the genes for more than 160 possible outer-membrane proteins in the \( P.\ aeruginosa \) genome, including 18 homologs of OprD though no homologs of the OmpF family of general-diffusion porins that are the major conduit of antibiotic uptake in most gram-negative bacteria. The contribution of these to outer membrane-permeability remains to be defined.

Two efflux systems have been described as having a role in intrinsic antibiotic resistance based on their apparent constitutive expression and the influence of mutations and inhibitors. The first of these to be studied was the MexAB-OprM system.\(^{16}\) This system is a prototype RND (resistance-nodulation-division) system with a cytoplasmic pump protein, MexB, a periplasmic linker protein, MexA, and an outer-membrane protein, OprM. Mutations that prevent expression of any or all of these genes results in hyper-susceptibility to quinolones, tetracyclines, chloramphenicol, sulfamethoxazole, trimethoprim, and some \( \beta \)-lactams, but not aminoglycosides, erythromycin, polymyxins or imipenem and other \( \beta \)-lactams.\(^{8,10}\) On the other hand, another efflux pump operon, MexX-MexY, in apparent collaboration with OprM,\(^{17}\) was recently discovered to have the capability to efflux many of the same substrates as MexAB-OprM but to have a primary role in intrinsic resistance to aminoglycoside antibiotics and erythromycin.\(^{17,18}\) Thus, mutation of MexXY led to increased susceptibility to these latter antibiotics, but affected a broader range of antibiotic classes only in a MexAB-deficient background.\(^{17,18}\) Consistent with this observation, knockouts of OprM, which is weakly expressed from a secondary promoter and which collaborates with both MexXY and MexAB, have a far greater effect than knockouts of either MexAB or MexXY.\(^{16,17}\)

\( P.\ aeruginosa \) strains produce an AmpC-like inducible chromosomal \( \beta \)-lactamase that can inactivate \( \beta \)-lactams by hydrolysis.\(^{2}\) Induction of this \( \beta \)-lactamase, which occurs upon exposure to some \( \beta \)-lactams, can result in increased resistance to the inducing and other \( \beta \)-lactams. However, not all \( \beta \)-lactams are strong inducers. Recently, two papers have examined the interplay of \( \beta \)-lactamases and efflux in determining resistance.\(^{9,10}\) The conclusions from these studies are that the susceptibility of \( P.\ aeruginosa \) to some \( \beta \)-lactams (e.g. ceftazidime, cefepime, piperacillin, aztreonam) is more strongly influenced by efflux, whereas susceptibility to others (imipenem, panipenem) is more strongly affected by the presence of \( \beta \)-lactamase, while a third group (ceftriaxone, meropenem, moxalactam) is influenced only by knock out of both efflux and \( \beta \)-lactamase. In contrast, in depressed mutants, knockout of efflux has no apparent effect.

### MUTATIONAL RESISTANCE

The major classes and types of antibiotics used in \( P.\ aeruginosa \) therapy are summarized in Table 1. We largely restrict discussion in this review to resistance mechanisms that affect these antibiotics, and especially to those mechanisms that are most commonly observed in the clinic (Table 2).

<table>
<thead>
<tr>
<th>Class</th>
<th>Agents</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>Ticarcillin,</td>
<td>Synergistic with aminoglycosides</td>
<td>May induce beta-lactamases in ( P.\ aeruginosa )</td>
</tr>
<tr>
<td></td>
<td>Carbenicillin,</td>
<td>against ( P.\ aeruginosa )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Piperacillin,</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Tazobactam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Ceftazidime,</td>
<td>Can be used as single agent</td>
<td>May induce beta-lactamases in ( P.\ aeruginosa )</td>
</tr>
<tr>
<td></td>
<td>Cefoperazone</td>
<td>against ( P.\ aeruginosa )</td>
<td></td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>Gentamicin,</td>
<td>Synergistic with beta-lactam</td>
<td>Narrow therapeutic/toxic ratio; penetrate poorly</td>
</tr>
<tr>
<td></td>
<td>Tobramycin,</td>
<td>antibiotics against ( P.\ aeruginosa )</td>
<td>into cerebrospinal fluid</td>
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<tr>
<td></td>
<td>Amikacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinolone</td>
<td>Ciprofloxacin</td>
<td>Can be given orally</td>
<td>Contraindicated in children under 16 years of age</td>
</tr>
<tr>
<td>Polymyxin</td>
<td>Colistin</td>
<td>Very active and little resistance</td>
<td>Possible toxicity concerns; used largely in cystic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>development</td>
<td>fibrosis patients.</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Imipenem,</td>
<td>Very broad spectrum of activity</td>
<td>May induce beta-lactamases; rapid development of</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
<td>against Gram-negative bacteria</td>
<td>resistance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>including ( P.\ aeruginosa )</td>
<td></td>
</tr>
</tbody>
</table>
Table 2  Major resistance mechanisms to anti-pseudomonal antibiotics

<table>
<thead>
<tr>
<th>Class</th>
<th>Agents</th>
<th>Resistance Mechanisms/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbenicillin,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Piperaclillin</td>
<td></td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Cefazidime,</td>
<td>Derepression of chromosomal β-lactamase. Overexpression of the MexAB-OprM multidrug efflux pump due to a NalB mutation. For the fourth generation cephalosporins cefepime and cefpirome, over expression of the MexCD-OprJ multidrug efflux pump due to an NfxB mutation.</td>
</tr>
<tr>
<td></td>
<td>Cefoperazone,</td>
<td></td>
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<tr>
<td></td>
<td>Cefepime,</td>
<td></td>
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<tr>
<td></td>
<td>Ceftirixime</td>
<td></td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>Gentamicin,</td>
<td>Overexpression of the MexXY efflux pump in impermeability type-resistance due to a mutation in the regulatory gene MexZ.</td>
</tr>
<tr>
<td></td>
<td>Tobramycin,</td>
<td>Plasmid-mediated production of modifying enzymes.</td>
</tr>
<tr>
<td></td>
<td>Amikacin</td>
<td></td>
</tr>
<tr>
<td>Quinolone</td>
<td>Ciprofloxacin</td>
<td>Target site mutations in the GyrA (or sometimes the GyrB) topoisomerase subunit; Overexpression of multidrug efflux pumps due to NalB, NfxB or NfxC mutations.</td>
</tr>
<tr>
<td>Polymyxin</td>
<td>Colistin</td>
<td>Outer membrane LPS changes due to PhoP/PhoQ regulatory mutations. No evidence this occurs in the clinic.</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Imipenem,</td>
<td>Loss of specific outer membrane porin channel. OprD; Reduction in levels of OprD due to an NfxC mutation that also upregulates multidrug resistance due to MexEF-OprN; For meropenem overexpression of the MexAB-OprM multidrug efflux pump due to a NalB mutation</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
<td></td>
</tr>
</tbody>
</table>

Multidrug resistance

Multidrug resistance can be caused by regulatory mutations naldB (mexR), nfxB or nfxC (mexT) leading to overexpression of three separate RND efflux systems, MexAB-OprM, MexCD-OprJ and MexEF-OprN respectively. The three classes of regulatory mutation cause distinct but substantially overlapping multiple antibiotic resistance profiles and nfxC mutations cause a reduction in levels of the imipenem porin OprD (leading to imipenem resistance) and coincident increases in the expression of MexEF-OprN.

Certain mutants, commonly designated as ‘intrinsically resistant’ to carbenicillin, show cross-resistance to a variety of structurally related antibiotics, produce only low levels of β-lactamases, and may indeed be overexpressing an efflux system; such isolates may be reasonably common. Detailed analysis of isolates from the Besançon University Hospital, France indicated that of 21 β-lactam resistant isolates, 10 overexpressed AmpC β-lactamase, while 11 overexpressed OprM, due in eight of the 11 isolates to mutations in the regulatory gene MexR.

Another study looking at 20 quinolone-resistant *P. aeruginosa* isolates from six Danish cystic fibrosis patients revealed 16 isolates with nfxB mutations and increased amounts of OprN and OprP in six and eight isolates respectively. Six isolates demonstrated efflux pump overexpression in the absence of gyrA mutations. However, it should be emphasized that this was a small study and stood in contrast to quinolone resistance observed from non-cystic fibrosis patients where mutations in gyrA and/or parC predominated.

Quinolones

Quinolone resistance is on the rise with reported frequencies of 12–20%. In most reported cases (with the prominent exception of the above-reported study) there was a missense mutation in the quinolone target (the gyrA subunit of DNA gyrase) at codon 83 (T83I), although other mutations are sometimes observed. Higher levels of resistance may involve additional mutations in gyrB (DNA gyrase B subunit) or parC (topoisomerase IV). These target site mutations affect susceptibility to all quinolones.

β-Lactams

As mentioned above, all *P. aeruginosa* strains have a chromosomal AmpC β-lactamase that is normally inducible but may be derepressed by mutation, or can be induced by certain β-lactams. Such inducers include clavulanate, normally used as a β-lactamase inhibitor (but not against class C, AmpC-like β-lactamases) and the antibiotic imipenem. Mutations resulting in β-lactamase derepression are the most common clinical cause of β-lactam resistance in *P. aeruginosa*. In enterobacterial species, such as *C. freundii* and *E. cloacae*, such mutations have been shown to result from inactivation of the ampD gene. However, in *P. aeruginosa*, inactivation of ampD results in only partial depression of β-lactamase at a frequency of 10⁻³, whereas full depression apparently requires an additional mutation and occurs at a frequency of 10⁻⁹. Other *P. aeruginosa* isolates express plasmid-encoded Class A or D β-lactamases with PSE-1 and PSE-4 being the most common types. There is some indication that...
specific β-lactamases, including the extended spectrum β-lactamase TEM-242–28 and the metalloenzyme, imipenemase \( \text{bla}_{\text{up}} \).29 can be acquired from or spread to enterobacterial isolates by horizontal transfer.

Imipenem and related carbapenems show unique resistance profiles. As excellent inducers of AmpC β-lactamase, they are not further influenced by mutational derepression of this chromosomal enzyme.2 Instead two types of mutants are observed in the clinic and the lab.30 One involves the loss by mutation of an outer membrane protein, OprD, that has been demonstrated to be an imipenem-specific porin (also used for uptake of meropenem but not other β-lactams2–195). Examination of the \( P \). aeruginosa genome sequence reveals 18 additional OprD homologs. However, there is no evidence for a role for these homologs in imipenem susceptibility. The second type of mutation arises from the co-regulation of \( \text{oprD} \) and the \( \text{mexEF-oprN} \) efflux operon, by \( \text{mexT (nfxC)} \) which positively regulates the former and negatively regulates the latter genes.30,31 Thus \( \text{nfxC} \) mutations lead to a major decrease in OprD levels while up-regulating MexEF-OprN, leading to imipenem and multidrug resistance respectively. Of significant concern is the recent observation that an eluate from siliconized latex urinary catheters can apparently induce this type of resistance.52

**Aminoglycosides**

Most large studies have indicated that around 10% of \( P \). aeruginosa isolates are aminoglycoside resistant, although higher levels of resistance occur in some studies for specific aminoglycosides.2 Although enzyme-mediated aminoglycoside resistance is observed, at least 50% and up to 90% of isolates appear to carry the ‘impermeability’ type resistance. A mutation that appears to correlate with this phenotype involves up-regulation of the partial RND system MexXY (named AmrAB in the reported study).32 Interestingly, over-expression of MexXY alone from the cloned gene did not result in resistance. The lack of overexpression of OprM in aminoglycoside-resistant18 mutants was promoted as evidence that an outer membrane efflux protein other than OprM might participate with MexXY in determining impermeability type aminoglycoside resistance.17

Another relevant form of aminoglycoside resistance is adaptive resistance.33 Such resistance is reversible, after a post-antibiotic effect, upon removal of selective pressure. It was shown to occur in an artificial biofilm34 and in a rabbit endocarditis model.35 The mechanism of adaptive resistance is not understood. However, we have demonstrated that the two-component regulatory system, PhoP-PhoQ, which responds to divalent cation concentrations, can regulate susceptibility to aminoglycosides as well as to polymyxin B and some cationic antimicrobial peptides.56 Interestingly, there is evidence that this system is activated in \( P \). aeruginosa isolates from cystic fibrosis patients, possibly because of the extensive aerosol usage of aminoglycosides and colistin (polymyxin E) in these patients.37

**IMPACT OF ANTIBIOTIC RESISTANCE ON \( P \). AERUGINOSA THERAPY**

The therapy of infections caused by \( P \). aeruginosa presents a daunting problem to medical practitioners. Not only are these bacteria intrinsically resistant to a wide range of antimicrobial agents, but also they are capable of developing resistance during therapy. Evolution of resistance during antimicrobial therapy is particularly problematic in cystic fibrosis, a condition characterized by chronic infection in which repeated therapeutic courses are prescribed. In fact, it has recently been shown that many strains from patients with cystic fibrosis (but not other conditions) are ‘hypermutable’ rendering them particularly likely to convert to antibiotic resistance during a course of antimicrobial therapy.30 The purpose of this section is to review the impact of antibiotic resistance on therapeutic strategies and special considerations in patients with cystic fibrosis.

**Infections in normal and compromised hosts**

\( P \). aeruginosa rarely causes infections in patients with intact immunity; when such infections occur, they are usually mild and associated with exposure to water.39 Serious infection with \( P \). aeruginosa is more commonly seen in the context of immunocompromising conditions. The critical role of neutrophils in host defense against \( P \). aeruginosa is graphically illustrated by the risk of bacteremia in neutropenic hosts;40 a complete ‘cure’ of neutropenic \( P \). aeruginosa bacteremia is unlikely to occur until the patient’s neutrophil count has returned to near normal.

A number of other conditions are associated with serious \( P \). aeruginosa infection. Intravenous drug users are at risk for \( P \). aeruginosa endocarditis.41 Patients who are receiving intensive care and mechanical ventilation via an endotracheal tube are at risk of developing ‘ventilator-associated pneumonia’ with \( P \). aeruginosa.42 \( P \). aeruginosa urinary tract infections are extremely rare in normal individuals and are usually associated with a structural anomaly of the urinary tract. Other serious infections caused by \( P \). aeruginosa include corneal ulceration with contact lens use and burn wound infections. In both cases, the barrier function of innate local defenses is disrupted and in burn wounds there are a range of secondary host defense defects, including impairment of neutrophil function. Thus in most cases, antibiotic therapy is complicated by the occurrence of \( P \). aeruginosa infections in patients with serious underlying ailments that impact on the ability of host defenses to assist antibiotics in clearing infections, combined with high intrinsic antibiotic resistance of this organism.

**Chronic infections in cystic fibrosis patients**

Patients with cystic fibrosis are at profound risk for respiratory tract infection with \( P \). aeruginosa.43 No single predisposing explanation for this propensity to infection has been found but a number of factors may conspire to enhance the risk of this peculiar infection. Despite the absence of a unifying explanation for the susceptibility to \( P \). aeruginosa infection, the vast majority of cystic fibrosis patients are ultimately infected. The bacteria appear to be acquired predominantly from the environment, but acquisition from other patients has been demonstrated.44 Infection with \( P \). aeruginosa, and particularly the mucoid variant, is associated with a poor prognosis; therefore physicians are eager to find ways to prevent acquisition of the organism as well as new strategies for eradication once it is acquired. Unfortunately, antibiotic
therapy is rarely if ever able to eliminate *P. aeruginosa* colonization and no preventative approaches have been found to be universally effective. Thus there is an urgent need to develop better strategies for treating infections once they are acquired.

There are certain special considerations in the use of antibiotics to treat chronic lung infections in patients with cystic fibrosis. First, patients with cystic fibrosis clear antibiotics including aminoglycosides, penicillins and trimethoprim-sulfamethoxazole, from their systems more rapidly than do other individuals,

57–59 and thus require extraordinarily high doses of most drugs to achieve therapeutic concentrations in serum or sputum.

45, 46 On the other hand, ciprofloxacin pharmacokinetics appear to be normal in patients with cystic fibrosis and dosing adjustments are not necessary.

50 Secondly, during the course of chronic respiratory tract infection in cystic fibrosis, strains of *P. aeruginosa* may undergo a range of phenotypic changes; they often become mucoid, non-motile and susceptible to the bactericidal effects of normal human serum.

53 Multiple different phenotypic variants of *P. aeruginosa* are often recovered from a single sputum culture (mucoid, non-mucoid, dwarf, pigmented, etc.). These variants may be genetically indistinguishable but very different in their susceptibility to antimicrobial agents although colonial appearance and antimicrobial susceptibility appear to vary independently of one another. It has been suggested that the mucoid exopolysaccharide of mucoid strains may interfere with the penetration of certain antibiotics to their site of action.

53 Thirdly, antimicrobial agents in cystic fibrosis must penetrate to endobronchial secretions (including sputum) in order to achieve an effect. Whereas aminoglycosides penetrate to and accumulate in cystic fibrosis sputum, their bioactivity is low.

54 In one study, eradication of *P. aeruginosa* from cystic fibrosis sputum was only achieved when sputum aminoglycoside concentrations exceeded the MIC of the infecting bacteria by 2-fold.

55 Furthermore, the mucoid exopolysaccharide secreted by mucoid strains may impede the penetration of certain antimicrobials to their site of action.

53 These factors may conspire to create an environment where suboptimal antimicrobial activity is achieved at the site of infection. Failure to attain a therapeutic success may therefore be a result of local effects in the lung of cystic fibrosis patients rather than antimicrobial resistance of the infecting bacteria.

**Development of resistance during therapy**

The above factors make antibiotic therapy in patients with cystic fibrosis very challenging. For this reason, we will use this situation to discuss antibiotic resistance development. Such patients are often chronically colonized with *P. aeruginosa* and each patient appears to persistently harbour the same strain, as determined by genetic fingerprinting.

51 Since patients are typically infected with high bacterial densities and are exposed frequently to antibiotics, one would predict that resistance would develop during therapy and this does indeed occur. Although resistance may develop during the course of therapy, reversion to susceptibility often occurs after the antimicrobial agent has been withdrawn. Since antimicrobial resistance fluctuates unpredictably among bacterial isolates from each patient, therapy must be guided by the susceptibility pattern of the bacterial isolate obtained immediately prior to initiation of therapy.

About a third of *P. aeruginosa* strains recovered from patients with cystic fibrosis have an unusual capacity to develop resistance to antibiotics; this feature has recently been described as ‘hypermutability’ and is caused by the mutator gene *mutS*.

58 It is likely that this inherent feature of cystic fibrosis strains, as well as the high bacterial load and the need for repeated prolonged course of therapy, contributes to the development of the resistance phenotype which is typical of cystic fibrosis isolates.

**THERAPY OF INFECTIONS WITH *P. AERUGINOSA***

**Antibiotic options**

Despite the fact that *P. aeruginosa* has high intrinsic resistance to antimicrobial agents, a number of drugs are available for treatment of infection. The major classes that have been used with success include aminoglycosides (such as gentamicin and tobramycin), semisynthetic penicillins (such as carbenicillin, ticarcillin and piperacillin) third generation cephalosporins (including ceftazidime and cefoperazone), quinolones (such as ciprofloxacin) and carbapenems (including imipenem and meropenem).

In addition to the conventional antibiotics listed above, newer agents are being developed to counter the problem of antimicrobial resistance. Small cationic peptides, either natural or synthetic, are active against many strains of *P. aeruginosa*. These agents are present throughout nature (including within human neutrophils) and provide hope for treating infections caused by strains that are resistant to currently available drugs.

56, 57 Human testing is underway, but these novel agents are not yet available for routine clinical use. Other agents to be considered are those which have not been thought to have useful antipseudomonal activity, e.g. the macrolides, but appear to improve the prognosis of patients with chronic *P. aeruginosa* pulmonary infections.

58 Such agents may indeed have a slow bactericidal effect on this organism.

**Combination therapy**

Therapy of serious infections caused by *P. aeruginosa*, usually consists of a combination of a semisynthetic penicillin, such as ticarcillin or piperacillin (with or without a β-lactamase inhibitor) and an aminoglycoside, such as tobramycin. These drugs have been shown to be synergistic in vitro against *P. aeruginosa*. Data on synergy is strain-specific, and there is debate regarding whether or not predictions about clinical efficacy can be made from in vitro observations. Nonetheless, most experts recommend that the two classes of drugs be used together for their possible synergistic effect.

Other agents that have proven to be effective in treating pulmonary exacerbations in cystic fibrosis include quinolones, newer β-lactams such ascefoperazone or ceftazidime, and carbapenems (imipenem or meropenem).

These agents may be given singly or in combination with an aminoglycoside, but they should be chosen in the light of
results from in vitro antimicrobial susceptibility testing. Combination therapy with agents other than penicillins and aminoglycosides has not been proven superior to use of single agents. Some of the newer agents, such as imipenem, induce β-lactamase activity and should not be given in combination with other β-lactams. Often, a clinical improvement may be seen even when antimicrobial agents are administered to which the infecting strain of *P. aeruginosa* is resistant in vitro.

**Inhalational therapy**

Local factors in the endobronchial space appear to interfere with the antibacterial activities of intravenously administered antibiotics. One strategy for enhancing the antibacterial effect of anti-Pseudomonal antibiotics has been to deliver the agents directly to the site of infection by the aerosol route. Inhaled antimicrobial agents used in patients with cystic fibrosis have included carbenicillin, gentamicin, cephaloridine, tobramycin, colistin, polymyxin B and amikacin alone or in combination with parenteral antibiotics. Indeed routine application of aerosolized colistin, sometimes in combination with other antibiotics, is often used in cystic fibrosis patients. Results from clinical trials of aerosolized colistin have been mixed as has been the quality of the study design. A recently reported placebo-controlled study evaluated the efficacy of inhaled tobramycin; patients received either 600 mg tobramycin per day intermittently or saline daily over a period of 24 weeks. Those who received the drug had a significant improvement in pulmonary function, a decrease in sputum density of *P. aeruginosa* and a decreased need for hospitalization. There was a small but non-significant increase in antibiotic-resistant isolates of *P. aeruginosa*. The use of inhaled antibiotics is an attractive option for maximizing the antibacterial effect at the site of infection. Long-term prospective studies will be required to determine the risk of acquisition of antibiotic resistance from this therapeutic strategy.

**STRATEGIES FOR PREVENTION OF EMERGENCE OF RESISTANCE**

**Combination therapy**

Since cross-resistance between major classes of anti-Pseudomonal antibiotics is unlikely to develop, it is common practice to treat serious infections with a combination of a β-lactam and an aminoglycoside. Not only do the drugs appear to exert a synergistic antibacterial effect, but they may also delay or prevent the emergence of resistance during therapy.

There are a few novel approaches under development in which the combination of agents would include an antibacterial agent and an inhibitor of a major resistance mechanism. The best example of this would be the use of a combination of β-lactamase inhibitor and β-lactam, although the β-lactamase inhibitors currently available in the clinic do not work well and a specific inhibitor of class C enzymes should be used. Another approach, being pursued by Microcide Pharmaceuticals (US) is the development of inhibitors of efflux pumps (e.g. MC-207, 110) as a method of potentiating the activity of fluoroquinolones, and possible other drugs. Similarly, the use of antimicrobial peptides that are able to break down the outer-membrane permeability barrier, but have little intrinsic antibiotic activity has been proposed as an approach to overcoming this type of intrinsic insusceptibility.

**Restriction of use**

Good antibiotic stewardship plays an important role in limiting the emergence of antibiotic resistance. Some antimicrobial agents should be reserved for therapy of infections when all other agents have failed. For instance, it is inadvisable to use a drug such as meropenem for the initial infection with *P. aeruginosa* in a patient with cystic fibrosis; its use should be reserved for infections when the infecting strain is resistant to the first-line drugs: a semisynthetic penicillin and an aminoglycoside. Some hospitals impose restrictions on certain antimicrobial agents to maximize the likelihood that they will be effective when a legitimate indication for their use arises.

**Prevention of infection**

Infection control plays an exceedingly important role in preventing the spread of antimicrobial-resistant bacteria within hospitals. Since *P. aeruginosa* is a hydrophilic organism, it can thrive in moist environments in the hospital and be disseminated from a common source. Common source outbreaks linked to contaminated hydrotherapy water have been documented on burn and surgical wards. Additional outbreaks have been linked to contaminated endoscopes.

*P. aeruginosa* can be transiently carried on the hands of medical and nursing personnel resulting in the spread of infection among patients. Principles of good infection control such as careful hand washing and barrier precautions should be utilized when dealing with patients with antibiotic-resistant bacteria. There is conflicting data about the spread of *P. aeruginosa* among patients with cystic fibrosis, but epidemics have been documented.

Immunization against *P. aeruginosa* would be a strategy for preventing acquisition and spread of antibiotic-resistant strains. Whereas several different vaccine strategies have been considered and tested, none has yet entered clinical use.

**CONCLUDING REMARKS**

It appears likely that antibiotic resistance will continue to be a problem in dealing with *P. aeruginosa* infections, since the fundamental issues underlying this problem (i.e. the condition of the patients that are prone to such infections, and the high intrinsic resistance of this bacterium) have remained constant. It appears unlikely that there will be a large number of novel effective antibiotics to impact on this problem in the next decade. Instead we need a combination of good management of those agents we have and the application of innovative therapeutic approaches, such as the use of anti-resistance strategies. In the longer term, the recent sequenc- ing of the genome of *P. aeruginosa* gives us hope to identify the basis for phenotypic resistance, and the discovery of novel targets for antimicrobial intervention.
References


