Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and impact on treatment

Robert E.W. Hancock,[1] David P. Speert[2]

Departments of Microbiology and Immunology,[1,2] Pediatrics,[2] University of British Columbia, Vancouver, British Columbia, Canada

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, mutational 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 (quinpristin/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,[4] 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 peptides[6] or mutations to create large channels in the outer membrane[7]) 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,[8] 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*[8]). 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.[5,8] 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

doi: 10.1054/drup.2000.0152, available online at http://www.idealibrary.com on IDEAL®

Table 1 **Antibiotics commonly used in the treatment of *Pseudomonas aeruginosa* infections**

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

majority of small channels and a minority of larger channels. The small channels are probably due to the N-terminal half forming an 8-stranded β-barrel with a small central water-filled channel that is too small to permit uptake of the above mentioned substrates.[13] 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 gluconate 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 knockout 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 β-lactams, but not aminoglycosides, erythromycin, polymyxins or imipenem and other β-lactams.[8,16] 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 β-lactamase that can inactivate β-lactams by hydrolysis.[2] Induction of this β-lactamase, which occurs upon exposure to some β-lactams, can result in increased resistance to the inducing and other β-lactams. However, not all β-lactams are strong inducers. Recently, two papers have examined the interplay of β-lactamases and efflux in determining resistance.[9,10] The conclusions from these studies are that the susceptibility of *P. aeruginosa* to some β-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 β-lactamase, while a third group (ceftriaxone, meropenem, moxalactam) is influenced only by knockout of both efflux and β-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 sumarized 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 2 **Major resistance mechanisms to anti-pseudomonal antibiotics**

Class	Agents	Resistance Mechanisms/Comments
Penicillin	Ticarcillin, Carbenicillin, Piperacillin	Derepression of chromosomal β-lactamase. Overexpression of the MexAB-OprM multidrug efflux pump due to a NalB mutation. Specific plasmid-mediated β-lactamases.
Cephalosporin	Ceftazidime, Cefoperazone, Cefepime, Cefpirome	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, overexpression of the MexCD-OprJ multidrug efflux pump due to an NfxB mutation.
Aminoglycoside	Gentamicin, Tobramycin, Amikacin	Overexpression of the MexXY efflux pump in impermeability type-resistance due to a mutation in the regulatory gene MexZ. Plasmid-mediated production of modifying enzymes.
Quinolone	Ciprofloxacin	Target site mutations in the GyrA (or sometimes the GyrB) topoisomerase subunit; Overexpression of multidrug efflux pumps due to NalB, NfxB or NfxC mutations.
Polymyxin	Colistin	Outer membrane LPS changes due to PhoP/PhoQ regulatory mutations. No evidence this occurs in the clinic.
Carbapenem	Imipenem, Meropenem	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

Multidrug resistance

Multidrug resistance can be caused by regulatory mutations *nalB (mexR)*, *nfxB* or *nfxC* (*mexT*) leading to overexpression of three separate RND efflux systems, MexAB-OprM, MexCD-OprJ and MexEF-OprN respectively.[19] 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.[20] 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*.[21]

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 OprJ in six and eight isolates respectively. Six isolates demonstrated efflux pump overexpression in the absence of *gyrA* mutations.[22] 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.[2,23]

Quinolones

Quinolone resistance is on the rise with reported frequencies of 12–20%.[24] In most reported cases (with the prominent exception of the above-reported[22] 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.[23,25] 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.[2,9,26] 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*.[2] 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^{-7}, whereas full depression apparently requires an additional mutation and occurs at a frequency of 10^{-9}.[27]

Other *P. aeruginosa* isolates express plasmid-encoded Class A or D β-lactamases with PSE-1 and PSE-4 being the most common types.[2,26] There is some indication that

specific β-lactamases, including the extended spectrum β-lactamase TEM-24[2,28] and the metalloenzyme, imipenemase bla_{IMP}[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-lactams[2,159]). 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 *oprD* and the *mexEF-oprN* efflux operon, by *mexT* (*nfxC*) which positively regulates the former and negatively regulates the latter genes.[30,31] Thus *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.[32]

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).[18] Interestingly, overexpression of MexXY alone from the cloned gene did not result in resistance. The lack of overexpression of OprM in aminoglycoside-resistant[18] 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 biofilm[34] 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.[36] 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.[38] 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 defences 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,[47–49] 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.[43] 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[51] but very different in their susceptibility to antimicrobial agents[52] 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*.[38] 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 meropenem and imipenem).

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 inections.[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*.[59] 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 as cefoperazone or ceftazidime, and carbapenems (imipenem or meropenem).[60,61] 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.[62]

Inhalational therapy

Local factors in the endobronchial space appear to interfere with the antibacterial activities of intravenously administered antibiotics.[54,55] 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.[63–67] 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.[65] Results from clinical trials of serosolized antibiotics have been mixed as has been the quality of the study design. A recently reported placebo-controlled study evaluated the efficacy of inhaled tobramycin;[67] 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.[26] 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.[68] 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.[69]

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.[70]

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.[44]

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[3] sequencing 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.

Received 16 June 2000;
Revised 4 July 2000;
Accepted 6 July 2000

Correspondence to: Robert E.W. Hancock, Center for Microbial Diseases and Host Defense Research, Department of Microbiology and Immunology, #300, 6174 University Blvd., University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3. Tel: 604 822 2682; Fax: 604 822 6041; E-mail bob@cmdr.ubc.ca

References

1. Hancock REW, Knowles D. Are we approaching the end of the antibiotic era? Editorial overview. Curr Opinion Microbiol 1998; 1: 493–494.
2. Hancock REW, Speert DP. Antibiotics for *Pseudomonas* and related infections. In: Dodge JA, Brock DJH, Widdicombe JH, eds. Cystic fibrosis – current topics. John Wiley and Sons Ltd, vol. 3: 1996; 245–266.
3. Stover KC, Pham XQ, Erwin AL et al. Complete genome sequence of *Pseudomonas aeruginosa*: a versatile organism. Nature 2000 (in press).
4. Flick MR, Cluff LE. *Pseudomonas* bacteremia: Review of 108 cases. Am J Med 1976; 60: 501–508.
5. Hancock REW. The bacterial outer membrane as a drug barrier. Trends Microbiol 1997; 5: 37–42.
6. Scott MG, Yan H, Hancock REW. Biological properties of structurally related α-helical cationic antimicrobial peptides. Infect Immun 1999; 67: 2005–2009.
7. Huang H, Hancock REW. The role of specific surface loop regions in determining the function of the imipenem-specific pore protein OprD of *Pseudomonas aeruginosa*. J Bacteriol 1996; 178: 3085–3090.
8. Nikaido H. Prevention of drug access to bacterial targets: Permeability barriers and active efflux. Science 1994; 264: 382–387.
9. Masuda N, Gotoh N, Ishii C, et al. Interplay between chromosomal β-lactamase and the MexAB-OprM efflux system in intrinsic resistance to β-lactams in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1999; 43: 400–402.
10. Nakae T, Nakajima A, Ono T, Saito K, Yoneyama H. Resistance to β-lactam antibiotics in *Pseudomonas aeruginosa* due to interplay between the MexAB-OprM efflux pump and β-lactamase. Antimicrob Agents Chemother 1999; 43: 1301–1303.
11. Nikaido H, Hancock REW. Outer membrane permeability of *Pseudomonas aeruginosa*. In: Sokatch JR, eds. The Bacteria: A treatise on structure and function. 1986; X: London: Academic Press, 145–193.
12. Bellido F, Martin NL, Siehnel RJ, Hancock REW. Reevaluation, using intact cells, of the exclusion limit and role of porin OprF in *Pseudomonas aeruginosa* outer membrane permeability. J Bacteriol 1992; 174: 5196–5203.
13. Brinkman FSL, Bains M, Hancock REW. The amino terminus of *Pseudomonas aeruginosa* outer membrane protein OprF forms channels in lipid bilayer membranes: correlation with a three-dimensional model. J Bacteriol 2000 (in press).
14. Wylie JL, Worobec EA. The OprB porin plays a central role in carbohydrate uptake in *Pseudomonas aeruginosa*. J Bacteriol 1995; 177: 3021–3026.
15. Huang H, Hancock REW. Genetic definition of the substrate selectivity of outer membrane porin protein OprD of *Pseudomonas aeruginosa*. J Bacteriol 1993; 175: 7793–7800.
16. Zhao Q, Li XZ, Srikumar R, Poole K. Contribution of outer membrane efflux protein OprM to antibiotic resistance in *Pseudomonas aeruginosa* independent of MexAB. Antimicrob Agents Chemother 1998; 42: 1682–1688.
17. Aires JR, Köhler TH, Nikaido H, Plésiat, P. Involvement of an active efflux system in the natural resistance of *Pseudomonas aeruginosa* to aminoglycosides. Antimicrob Agents Chemother 1999; 43: 2624–2628.
18. Westbrock-Wadman S, Sherman DR, Hickey MJ et al. Characterization of a *Pseudomonas aeruginosa* efflux pump contributing to aminoglycoside impermeability. Antimcrob Agents Chemother 1999; 43: 2975–2983.
19. Germ M, Yoshihara E, Yoneyama H, Nakae T. Interplay between the efflux pump and the outer membrane permeability barrier in fluorescent dye accumulation in *Pseudomonas aeruginosa*. Biochem Biophys Res Communic 1999; 261: 452–455.
20. Nikaido H. Antibiotic resistance caused by gram-negative multidrug efflux pumps. Clin Infect Dis 1998; 27: S32–S41.
21. Ziha-Zarifi I, Llanes C, Köhler T, Pechere JC, Plésiat P. In vivo emergence of multidrug-resistant mutants of *Pseudomonas aeruginosa* overexpressing the active efflux system MexA-MexB-OprM. Antimicrob Agents Chemother 1999; 43: 287–291.
22. Jalal S, Ciofu O, Høiby N, Gotoh N, Wretlind B. Molecular mechanisms of fluoroquinolone resistance in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. Antimicrob Agents Chemother 2000; 44: 710–712.
23. Mouneimné H, Robert J, Jarlier V, Cambau E. Type II topoisomerase mutations in Ciprofloxacin-resistant strains of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1999; 43: 62–66.
24. Anonymous. United States geographic bacteria susceptibility patterns. 1997 ASCP Susceptibility Testing Group. Diagnostic Microbiol Infect Dis 1999; 35: 143–151.
25. Takenouchi T, Sakagawa E, Sugawara M. Detection of gyrA mutations among 335 *Pseudomonas aeruginosa* strains isolated in Japan and their susceptibilities to fluoroquinolones. Antimicrob Agents Chemother 1999; 43: 406–409.
26. Livermore DM, Chen HY. Potentiation of β-lactams against *Pseudomonas aeruginosa* strains by Ro 43–1356, a bridged monobactam inhibitor of AmpC β-lactamases. J Antimicrob Chemother 1997; 40: 335–343.
27. Langaee TY, Gagnon L, Huletsky A. Inactivation of the *ampD* gene in *Pseudomonas aeruginosa* leads to moderate-basal-level and hyperinducible AmpC β-lactamase expression. Antimicrob Agents Chemother 2000; 44: 583–589.
28. Marchandin H, Jean-Pierre H, De Champs C et al. Production of a TEM-24 plasmid-mediated extended-spectrum β-lactamase by a clinical isolate *of Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2000; 44: 213–216.
29. Laraki N, Galleni M, Thamm I et al. Structure of In31, a *bla*$^{IMP-}$ containing *Pseudomonas aeruginosa* integron phyletically related to In5, which carries an unusual array of gene cassettes. Antimicrob Agents Chemother 1999; 43: 890–901.
30. Kohler T, Michea-Hamzehpour M, Epp SF, Pechere JC. Carbapenem activities against *Pseudomonas aeruginosa*: Respective contributions of OprD and efflux systems. Antimicrob Agents Chemother 1999; 43: 424–427.

31. Ochs, MM, McCusker MP, Bains M, Hancock REW. Negative regulation of the *Pseudomonas aeruginosa* outer membrane porin OprD selective for imipenem and basic amino acids. Antimicrob Agents Chemother 1999; 43: 1085–1090.
32. Martínez-Martínez L, Pascual A, Del Carmen Conejo M, Picabea L, Perea EJ. Resistance of *Pseudomonas aeruginosa* to imipenem induced by eluates from siliconized latex urinary catheters is related to outer membrane protein alterations. Antimicrob Agents Chemother 1999; 43: 397–399.
33. Karlowsky JA, Zelenitsky SA, Zhanel GG. Aminoglycoside adaptive resistance. Pharmacotherapy 1997; 17: 549–555.
34. Brooun A, Liu S, Lewis K. A dose-response study of antibiotic resistance in *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother 2000; 44: 640–646.
35. Xiong YQ, Caillon J, Kergueris MF et al. Adaptive resistance of *Pseudomonas aeruginosa* induced by aminoglycosides and killing kinetics in a rabbit endocarditis model. Antimicrob Agents Chemother 1997; 41: 823–826.
36. McFarlane ELA, Kwasnicka A, Hancock REW. Role of *Pseudomonas aeruginosa* in resistance to antimicrobial peptides and aminoglycosides. Microbiol 2000; In press.
37. Ernst RK, Yi EC, Guo L et al. Specific lipopolysaccharide found in cystic fibrosis airway *Pseudomonas aeruginosa*. Science 1999; 286: 1561–1565.
38. Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science 2000; 288: 1251–1253.
39. Salmen P, Dwyer DM, Vorse H, Kruse W. Whirlpool-associated *Pseudomonas aeruginosa* urinary tract infections. JAMA 1983; 250: 2025–2026.
40. Speert DP. *Pseudomonas aeruginosa* – phagocytic cell interactions. In: Friedman H, Campa M, Bendinelli M, Friedman H, eds. *Pseudomonas aeruginosa* as an opportunistic pathogen. New York: Plenum Press, 1993; 163–168.
41. Jackson GG. Infective endocarditis caused by *Pseudomonas aeruginosa*. In: Baltch AL, Smith RP, eds. *Pseudomonas aeruginosa* infections and treatment. New York: Marcel Dekker, Inc., 1994; 129–158.
42. Kollef MH. Ventilator-associated pneumonia: the importance of initial empiric antibiotic selection. Infect Med 2000; 17: 265–268.
43. Speert DP. *Pseudomonas aeruginosa* infections in patients with cystic fibrosis. In: Baltch AL, Smith RP, eds. *Pseudomonas aeruginosa* infections and treatment. New York: Marcel Dekker, Inc., 1994; 183–236.
44. Cheng K, Smyth RL, Govan JRW, et al. Spread of β-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. Lancet 1996; 348: 639–642.
45. Prandota J. Drug dispositionin cystic fibrosis: progress in understanding pathophysiology and pharmacokinetics. Pediatr Infect Dis J 1987; 6: 1111–1126.
46. de Groot R, Smith AL. Antibiotic pharmacokinetics in cystic fibrosis: differences and clinical significance. Clin Pharmacokinet 1987; 13: 228–253.
47. Levy J, Smith AL, Koup JR, Williams-Warren J, Ramsey B. Disposition of tobramycin in patients with cystic fibrosis: A prospective controlled study. J Pediatr 1984; 105: 117–124.
48. de Groot R, Hack BD, Weber A, et al. Pharmacokinetics of ticarcillin in patients with cystic fibrosis: a controlled prospective study. Clin Pharmacol Ther 1990; 49: 73–78.
49. Hutabarat RM, Unadkat JD, Sahajwalla C, McNamara S, Ramsey B, Smith AL. Disposition of drugs in cystic fibrosis. I. Sulfamethoxazole and trimethoprim. Clin Pharmacol Ther 1991; 49: 402–409.
50. Davis RL, Koup JR, Williams-Warren J, et al. Pharmacokinetics of ciprofloxacin in cystic fibrosis. Antimicrob Agents Chemother 1987; 31: 915–919.
51. Mahenthiralingam E, Campbell ME, Foster J, Lam JS, Speert DP. Random amplified polymorphic DNA typing of *Pseudomonas aeruginosa* isolates recovered from patients with cystic fibrosis. J Clin Microbiol 1996; 34: 1129–1135.
52. Thomassen MJ, Demko CA, Boxerbaum B, Stern RC, Kuchenbrod PJ. Multiple isolates of *Pseudomonas aeruginosa* with differing antimicrobial susceptibility patterns from patients with cystic fibrosis. J Infect Dis 1979; 140: 873–880.
53. Slack MPE, Nichols WW. The penetration of antibiotics through sodium alginate and through the exopolysaccharide of a mucoid strain of *Pseudomonas aeruginosa*. Lancet 1981; 5: 502–503.
54. Levy J, Smith AL, Kenny MA, et al. Bioactivity of gentamicin in purulent sputum from patients with cystic fibrosis or bronchiectasis: Comparison with activity in serum. J Infect Dis 1983; 148: 1069–1076.
55. Mendelman PM, Smith AL, Levy J, et al. Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum. Am Rev Respir Dis 1985; 132: 761–765.
56. Hancock REW, Lehrer R. Cationic peptides: a new source of antibiotics. Trends Biotechnol 1998; 16: 82–88.
57. Hancock REW. The therapeutic potential of cationic peptides. Expert Opin Invest Dis 1998; 7: 167–174.
58. Tateda K, Ishii Y, Matsumoto T, et al. Direct evidence for antipseudomonal activity of macrolides: Exposure-dependent bactericidal activity and inhibition of protein synthesis by erythromycin, clarithromycin and azithromycin. Animicrob Agents Chemother 1996; 40: 2271–2275.
59. Craig WA, Ebert SC. Antimicrobial therapy in *Pseudomonas aeruginosa* infections. In: Baltch AL, Smith RP, eds. *Pseudomonas aeruginosa* infections and treatment. New York: Marcel Dekker, Inc., 1994; 441–518.
60. LeBel M. Fluoroquinolones in the treatment of cystic fibrosis: a critical appraisal. Eur J Clin Microbiol Infect Dis 1991; 10: 316–324.
61. De Boeck K, Smet M, Eggermont E. Treatment of *Pseudomonas* lung infection in cystic fibrosis with piperacillin plus tobramycin versus ceftazidime monotherapy: preliminary communication. Ped Pulmonol 1989; 7: 171–173.
62. Zabner R, Quinn JP. Antimicrobials in cystic fibrosis: emergence of resistance and implications for treatment. Sem Resp Infect 1992; 7: 210–217.
63. Hodson ME. Antibiotic treatment – aerosol therapy. Chest 1988; 94(suppl): 156S–162S.
64. Wall MA, Terry AB, Eisenberg J, McNamara M, Cohen R. Inhaled antibiotics in cystic fibrosis. Lancet 1983; 11: 1325–1326.
65. Littlewood JM, Miller MG, Ghoneim AT, Ramsden CH. Nebulised colomycin for early Pseudomonas colonisation in cystic fibrosis. Lancet 1985; i:865.
66. Smith AL, Ramsey BW. Aerosol administration of antibiotics in patients with cystic fibrosis. Report Ped Infect Dis 1994; 4: 23–24.
67. Ramsey BW, Pepe MS, Quan JM, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. N Engl J Med 1999; 340: 23–30.

68. Reneau TE, Leger R, Flamme EM, et al. Inhibitors of efflux pumps in Pseudomonas aeruginosa potentiate the activity of the fluoroquinolone antibacterial levofloxacin. J Med Chem 1999; 42: 4928–4931.
69. Zhang L, Benz R, Hancock, REW. Influence of proline residues on the antibacterial and synergistic activities of α-helical peptides. Biochemistry 1999; 38: 8102–8111.
70. Beck-Sagué CM, Banerjee SN, Jarvis WR. Epidemiology and control of *Pseudomonas aeruginosa* in U.S. hospitals. In: Baltch AL, Smith RP, eds. *Pseudomonas aeruginosa* infections and treatment. New York: Marcel Dekker, Inc., 1994; 51–72.