Challenges in Identifying New Antimicrobial Agents Effective for Treating Infections with *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

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*Acinetobacter baumannii* and *Pseudomonas aeruginosa* are gram-negative pathogens that target immunocompromised patients. They express a variety of determinants that confer resistance to a broad array of antimicrobial agents. Mechanisms of resistance include impaired entry through the bacterial outer membrane, production of antibiotic-modifying enzymes, active efflux, and target mutations that reduce antimicrobial affinity. It has been a challenge to identify new agents that have activity against the more resistant variants of these species. Doripenem is a carbapenem in phase 3 trials that has excellent activity against *P. aeruginosa* and *A. baumannii*. However, it lacks activity against strains that express resistance to the currently available carbapenems. Tigecycline is a newly licensed glycylcycline that lacks activity against *P. aeruginosa* but has encouraging activity against many *A. baumannii* isolates. Resistance to tigecycline can emerge during therapy, however, and is due to expression of multidrug efflux pumps.

Antimicrobial resistance is a growing problem in modern hospitals. The increasing severity of illness and compromised immunity of patients treated for cancer and other illnesses lead to frequent use of broad-spectrum antimicrobial agents. These circumstances create environments in which resistant pathogenic bacteria have a significant survival advantage. In this setting, several multidrug-resistant pathogens have become particularly prominent as causes of nosocomial infections. Among gram-positive species, methicillin-resistant staphylococci and multidrug-resistant enterococci now constitute ~60% and 30% of *Staphylococcus aureus* and enterococcal isolates from infections in patients housed in intensive care units (ICUs) [1]. Among gram-negative species, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have developed resistance to multiple antimicrobial agents with the greatest frequency, in some cases expressing resistance to all clinically available compounds. Here, I focus on the clinical importance and emerging resistance of these 2 pathogens and explore the potential for new targets for antimicrobial therapy.

**CLINICAL SPECTRUM OF INFECTIONS WITH *P. AERUGINOSA* AND *A. BAUMANNII***

*P. aeruginosa* is a true opportunistic pathogen that causes infections in a variety of clinical settings. In relatively rare circumstances, *P. aeruginosa* is a prominent pathogen in infections in which the host is not compromised in some way. Examples of such infections include soft tissue infections resulting from punctures of the lower extremities, occurring through worn old tennis shoes, or folliculitis resulting from bathing in hot tubs [2]. In most cases, however, *P. aeruginosa* infections occur in patients who have been compromised in some way. In those with diabetes and in other immunocompromised patients, *P. aeruginosa* causes malignant external otitis, an invasive and necrotizing infection of the external ear canal that can result in osteomyelitis, nerve compromise, and even death in infected patients [3]. In patients with cystic fibrosis, *P. aeruginosa* commonly colonizes and infects the lower...
respiratory tract [4]. In hospitalized patients, particularly those who are intubated and undergoing mechanical ventilation in the ICU, P. aeruginosa is a prominent cause of ventilator-associated pneumonia (VAP) [1]. It also figures prominently in infections of indwelling devices, such as urinary and intravenous catheters.

P. aeruginosa has for years been a major influence in the antimicrobial treatment of patients with greatly depressed neutrophil counts (neutropenia) resulting from chemotherapy for the treatment of various malignancies. This influence stems from early studies of cancer chemotherapy, in which it was found that 50% of patients with neutropenia who had bacteremia due to P. aeruginosa were dead by the time the P. aeruginosa was detected on culture in the microbiology laboratory [5]. This dramatic finding was instrumental in 2 subsequent policy dictates that had major influences on hospital practice and on the development of new antimicrobial agents by the pharmaceutical industry. The first was the practice of instituting antimicrobial therapy for patients with neutropenia at the time the fever was recognized (rather than waiting for culture results). The second was the practice of ensuring that regimens used to treat febrile patients with neutropenia contained at least 1 antimicrobial agent with in vitro activity against P. aeruginosa. P. aeruginosa remains an important cause of febrile neutropenia, although considerable geographic variation in the incidence of such infections exists [6]. Fortunately, unlike in the early days, we now have several classes of antimicrobial agents with potent activity against (susceptible) P. aeruginosa (extended-spectrum penicillins and cephalosporins, carbapenems, aztreonam, fluoroquinolones, and aminoglycosides).

Acinetobacter species (predominantly A. baumannii) cause infection almost exclusively in the hospital setting and particularly in patients in ICUs [7]. It causes VAP and, in outbreak situations, can be very difficult to control in units with large numbers of patients undergoing ventilation [7]. It also causes soft tissue infections, urinary tract infections, catheter-associated bloodstream infections, and primary bacteremia [8]. Although some have raised doubts about the true impact of A. baumannii on the clinical course of VAP [9], most clinicians who have confronted this pathogen with some frequency have learned to respect its ability to cause significant morbidity.

**MECHANISMS OF RESISTANCE IN P. AERUGINOSA AND A. BAUMANNII**

The growth of molecular biological techniques and knowledge over the past 2 decades has solved many of the mysteries surrounding the expression of antibiotic resistance by P. aeruginosa. We have come to understand that P. aeruginosa possesses more tools for defying the activity of antimicrobial agents than virtually any other microorganism (table 1). Chief among the mechanisms for evolving resistance is the capacity that P. aeruginosa has to dramatically reduce access of the antibiotic to its target. Reduced access to the target not only raises the MICs in and of itself, it also amplifies the level of resistance conferred by other mechanisms, either intrinsic, mutational, or acquired.

Compared with that of bacteria such as Escherichia coli, the major outer membrane porin of P. aeruginosa (OprF) transports solutes at a rate that is slower by 2 orders of magnitude [10]. This slow transport is due to the protein’s ability to assume 2 conformations, one resulting in a very narrow and restrictive channel and the other resulting in a channel that is wide open. The mechanisms for regulating which conformation OprF takes have not been well defined. Some outer membrane proteins are very important conduits for antimicrobial entry. In particular, P. aeruginosa expresses OprD2 (outer membrane protein D2), a porin that is the major mechanism by which imipenem enters the cell [11]. As a zwitterion (an agent with no net charge), imipenem enters the periplasmic space rapidly through this porin, allowing it to avoid the rather weak hydrolyzing activity of P. aeruginosa’s chromosomally encoded AmpC β-lactamase. Unfortunately, P. aeruginosa possesses at least 2 mechanisms by which OprD2 can be down-regulated, resulting in restricted imipenem entry that, in combination with high-level expression of AmpC (which is induced by imipenem exposure), confers clinically significant levels of resistance to this antibiotic. Down-regulation of OprD2 is not rare. As many as 38% of patients treated with imipenem for pneumonia due to P. aeruginosa will have a resistant isolate recovered before the end of therapy [12].

In addition to slow entry, P. aeruginosa has substantial mechanisms for removing toxic compounds from proximity to their targets. The most important of these are the so-called RND (resistance-nodulation–cell division) 3-component pumps. Survey of the P. aeruginosa genome reveals 10 operons that encode likely RND-type multidrug efflux pumps, and several other genes likely encode pumps of other classes [13]. Seven of the RND-type pumps have been characterized, with 6 of them active against antimicrobial agents [14]. Among the more common substrates for such pumps are the fluoroquinolones,

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Table 1. Mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa* and Acinetobacter baumannii.
but they also expel aminoglycosides, β-lactam, tetracyclines, chloramphenicol, and macrolides, among others. Only one of these pumps, MexAB-OprM, is expressed constitutively [15]. This pump is active against a variety of compounds, including fluoroquinolones and many β-lactams, but is less effective at pumping out fourth-generation cephalosporins [16]. A second pump, MexCD-OprF, can become up-regulated and result in resistance to fourth-generation cephalosporins [16]. Increased expression of a third pump, MexEF-OprD, is associated with decreased expression of OprD2 [17]. Therefore, although no pump yet identified has counted imipenem among its substrates, increased expression of MexEF-OprD will confer resistance to imipenem through its action on OprD2. MexXY-OprM is a fourth pump whose substrates include the aminoglycosides [18]. These multiple mechanisms for preventing toxic compounds from entering the cell in significant concentrations make P. aeruginosa an ideal microorganism for surviving in toxic environments, including the antimicrobial-rich modern hospital environment.

P. aeruginosa clinical isolates also express a variety of enzymes designed to inactivate antibiotics as they approach their targets. Some of these, such as the chromosomal AmpC β-lactamase, are intrinsic to the species [19]. Many β-lactamases are acquired, often with mutations that extend their spectra. P. aeruginosa clinical isolates have been reported to express all 4 Ambler classes (a commonly used β-lactam classification scheme based on β-lactamase structure [20]) of β-lactamase, including metallo-enzymes that are active against the most stable of the β-lactam classes, the carbapenems [21]. P. aeruginosa also expresses a variety of different aminoglycoside-modifying enzymes, the molecules that acylate, adenylate, or dephosphorylate the compounds and eliminate their effectiveness [22]. These acquired genes may be found on transferable plasmids and are often incorporated within integrons along with other resistance determinants.

When modifying enzymes are not available, and when efflux or decreased entry does not result in a MIC sufficiently high to avoid inhibition in the clinical setting, P. aeruginosa is quite capable of acquiring mutations within cellular target genes to confer sufficient levels of resistance. This mechanism is particularly apparent with resistance to fluoroquinolones, which can be conferred at high levels by mutations within the cellular topoisomerase genes [23]. The stepwise and incremental nature of this type of resistance has promoted the concept of the “mutant prevention concentration” [24], whereby the most potent fluoroquinolone should always be used in the hope that the concentrations of antibiotic at the site of contact will exceed the MIC required by the common first-step mutants. This concept has been particularly promoted for the treatment of Streptococcus pneumoniae infections by the newer, more potent fluoroquinolones gatifloxacin and moxifloxacin and, to a lesser extent, levofloxacin. Unfortunately, although the newer fluoroquinolones are truly more potent than ciprofloxacin against S. pneumoniae, the reverse is true against P. aeruginosa. One might suspect, then, that widespread use of newer fluoroquinolones would promote the emergence of more-resistant P. aeruginosa. In fact, since levofloxacin gained widespread use in the late 1990s, rates of fluoroquinolone resistance have soared in many ICUs [1]. In the most recent data available on susceptibility of strains isolated from patients with infections in ICUs, 29.5% of P. aeruginosa isolates expressed resistance to the fluoroquinolones [1].

Although this phenomenon has not yet described in P. aeruginosa, a variety of Enterobacteriaceae isolates have recently been found to express qnr genes. These genes encode proteins that “protect” topoisomerase from the activity of the fluoroquinolones [25, 26]. Although they do not by themselves provide a high level of resistance to fluoroquinolones, they can enhance the level of resistance that is conferred by topoisomerase point mutations, similar to the expression of efflux pumps. Given the ready transfer of genes among and between gram-negative genera, it seems likely that these resistance determinants will eventually appear in P. aeruginosa.

Finally, a discussion of resistance in P. aeruginosa would be incomplete without an acknowledgement of the importance of the biofilms within which this species resides in many cases of clinical infection. By mechanisms that remain under intense investigation, bacteria within biofilms often express decreased susceptibility to antibiotics, compared with that of bacteria grown planktonically [27]. Infection in the presence of foreign bodies is virtually synonymous with biofilm involvement. Therefore, one can assume that the majority of nosocomial P. aeruginosa infections (VAP, catheter-associated urinary tract infection, and catheter-associated bloodstream infection) involve biofilms. Moreover, the P. aeruginosa strains that cause infection of the lower respiratory tracts of patients with cystic fibrosis are generally heavily prone to form biofilms.

A. baumannii has been an “organism of interest” in nosocomial infections for a much shorter period than has P. aeruginosa, and so less is known about the mechanisms of resistance for this species. Nevertheless, much has been learned over the past decade. Like P. aeruginosa, A. baumannii has been found to express a variety of different β-lactamases, including metallo-
enzymes that confer resistance to carbapenems [21]. Moreover, 2 RND-type multidrug efflux pumps have now been described in Acinetobacter species (AdeABC in A. baumannii and AdeDE in Acinetobacter genomic DNA group 3) [28, 29]. Like most bacteria, A. baumannii forms biofilms, which certainly complicates the treatment of device-associated infections [30].

NEW ANTIBIOTICS WITH ACTIVITY AGAINST P. AERUGINOSA AND A. BAUMANNII

The preceding discussion indicates the daunting challenge of developing new antibiotics with activity against P. aeruginosa and A. baumannii. Not only must the antibiotic be active against its intended target, but it must be able to enter the cells, remain long enough to interact with the target, and avoid expulsion by one of the multidrug efflux systems. Moreover, the major focus of antimicrobial resistance concerns in the 1990s was gram-positive bacteria such as methicillin-resistant staphylococci and vancomycin-resistant enterococci. Effective agents have been developed against these pathogens, and some are nearing approval by the US Food and Drug Administration. However, most tend to be restricted in their activities against gram-negative bacteria. Finally, many large pharmaceutical firms have abandoned their programs for antimicrobial development over the past decade, largely because of concerns that development costs exceed predicted profitability of injectable antimicrobial agents [31]. Perhaps not surprisingly, therefore, the number of antibiotics with activity against resistant gram-negative species under development is few.

Doripenem is an injectable carbapenem being developed by Peninsula Pharmaceuticals [32, 33]. It has activity against gram-positive organisms similar to that of imipenem and activity against gram-negative organisms similar to that of meropenem. In a recent in vitro study examining doripenem’s activity in comparison with those of other agents against well-characterized P. aeruginosa strains, doripenem exhibited the lowest MICs of all the agents tested (aztreonam, cefepime, ceftazidime, doripenem, ertapenem, imipenem, meropenem, and piperacillin-tazobactam) [34]. Moreover, exposure to doripenem resulted in the smallest percentage of 8 tested strains yielding resistant mutants, and those mutants, in general, required lower multiples of the original MIC than did mutants resulting from exposure to the other β-lactam agents [34]. Thus, it would appear that doripenem is less likely than other carbapenems to select for intrinsic resistant mutants of P. aeruginosa. However, it should be noted that doripenem is no less susceptible to hydrolysis by metallo–β-lactamases than are the other carbapenems.

Doripenem, imipenem, and meropenem are equally active against carbapenemase-negative A. baumannii strains, but none of the carbapenems was active against A. baumannii strains expressing plasmid-mediated carbapenemases [35]. Doripenem is stable enough in the presence of renal dehydropeptidase I that it need not be coadministered with a dehydropeptidase I inhibitor such as cilastatin. Pharmacokinetic studies have supported a 3-times-daily dosing schedule.

Doripenem is currently undergoing 6 phase 3 trials for treatment of complicated urinary tract infection (including pyelonephritis), complicated intra-abdominal infection, and pneumonia (including VAP). A phase 2 trial that enrolled 121 patients for the treatment of complicated urinary tract infections and tested doses of 250 or 500 mg 3 times daily was completed in 2003. The results of this trial supported moving forward with phase 3 studies.

Tigecycline is the first member of a new class of antimicrobial agents (glycylcyclines) (figure 1). It was recently approved by the US Food and Drug Administration for the treatment of complicated skin and soft tissue infections and complicated intra-abdominal infections, on the basis of non-inferiority to comparator agents in 2 trials for each indication [36, 37]. Tigecycline is broadly active in vitro against a range of gram-positive, gram-negative, and anaerobic pathogens [38]. In particular, tigecycline demonstrates activity against multidrug-resistant strains of A. baumannii [39]. However, not enough patients infected with A. baumannii were included in clinical trials of tigecycline to gain a clinical indication for use to treat these infections. Tigecycline does not have clinically significant activity against P. aeruginosa or Proteus species [38]. This lack of activity likely occurs because it appears to be a substrate for RND-type pumps constitutively expressed in these species [40]. Tigecycline is also a substrate for the RND-type pumps encoded by Enterobacteriaceae species, against which it is generally active [41–43]. Recently, reduced susceptibility of S. aureus to tigecycline has been attributed to the activity of a multidrug and toxin extrusion (MATE) pump [44].

Tigecycline is extensively distributed into many tissues, resulting in a prolonged half-life that justifies twice-daily dosing [45]. Fifty-nine percent of a tigecycline dose is excreted through the liver, and 33% is excreted through the kidney. In patients with severe hepatic impairment (Child’s class C), the normal dose of tigecycline (100-mg initial dose, then 50 mg twice daily) should be reduced to 25 mg twice daily after the normal loading dose. No adjustments are required for any level of renal impairment.

Whether tigecycline becomes a viable therapy for severe A. baumannii infections remains to be determined. The existence of at least 2 RND-type pumps in A. baumannii suggests that resistance may emerge during therapy (as it does to virtually all other antimicrobial agents), although it is not clear at this point whether tigecycline is a substrate for either of the 2 A. baumannii pumps thus far described.

No other antimicrobial agents with clinically significant activity...
against either P. aeruginosa or A. baumannii are at or beyond phase 2 at the time of writing of this article, nor are there any inhibitors of promising new targets, such as peptide deformylase, that have activity against these 2 pathogens. Potentially promising areas for future development include peptide antibiotics and inhibitors of common resistance mechanisms. Presently available peptide antibiotics (colistin [polymyxin E] and polymyxin B) do have activity against many A. baumannii and P. aeruginosa strains. However, they have a reputation (perhaps a bit exaggerated) for nephrotoxicity and neurotoxicity, and resistance can emerge during therapy via alterations in membrane charge. At present, no peptide antibiotics are being developed for the treatment of serious gram-negative infections by systemic administration.

Potentially promising strategies for the inhibition of resistance mechanisms include developing novel broad-spectrum β-lactamase inhibitors. The appeal of developing such inhibitors lies in the remarkable clinical success of the currently available β-lactam–β-lactamase inhibitors ampicillin-sulbactam, ticarcillin–clavulanic acid, and piperacillin-tazobactam. These agents are all limited, however, by the lack of inhibitor activity against some increasingly common enzymes, including AmpC enzymes and metallo-enzymes. The divergent structures of the different β-lactamase classes make designing a broad-based inhibitor challenging. Moreover, the multitude of other resistance mechanisms expressed by P. aeruginosa, for example, creates some caution about the long-term potential of such a strategy.

The importance of efflux as a mechanism of resistance to a variety of antimicrobial agents makes developing efflux inhibitors an attractive strategy. Such efforts are complicated by the fact that multidrug-resistant bacteria have the capacity to express a variety of different efflux pumps [48]. Expression of these alternative pumps may thwart the effect of an inhibitor of a specific efflux pump. Because human cells use efflux pumps as well, the development of a truly broad-spectrum efflux pump inhibitor would theoretically increase the risk of toxicity. Despite these challenges, continued research into development of inhibitors of resistance mechanisms is clearly needed.

CONCLUSIONS

The challenge of developing new agents for the treatment of infection with multidrug-resistant gram-negative species derives from the fact that these pathogens are uniquely suited to survival in toxic environments. They express a wide array of resistance mechanisms that are, in some cases, not specific to the antibiotic being administered but are general mechanisms for reducing exposure of the bacterium to toxic environmental materials. Because any new antimicrobial agent that is developed will certainly be toxic to the target organism, there is a real chance that one of the general mechanisms will help the bacterium to express resistance de novo or to evolve resistance under selective pressure. Nevertheless, the recent explosion in bacterial genetic information and our evolving ability to design and manipulate chemicals offers promise that new targets will be identified and new inhibitors will be developed. Until they are developed, however—and, in fact, even after they are developed—we must remain mindful that use of large volumes and indiscriminate use of any antimicrobial agents will inevitably lead to resistance. As such, continued emphasis on effective infection control measures and judicious and parsimonious use of available antimicrobial agents will remain mainstays in any reasonable strategy to maximize the usefulness of available antimicrobial agents.

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