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Unmet medical needs in antibacterial therapy

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ABSTRACT

The innate and evolutionary resourcefulness of bacterial pathogens virtually guarantees that there will always be important areas in which antimicrobial therapy can be improved. Current areas of need, or ones that are anticipated to be problematic in the near future include nosocomial infections caused by multi-resistant Gram-negative bacteria, where the variety and prevalence of multidrug efflux pumps provides a particular challenge to the designers of new drugs. In the community setting, the current prevalence of ampicillin and trimethoprim-sulfamethoxazole resistance, and the growing prevalence of fluoroquinolone resistance in *Escherichia coli* portend a need for new classes of oral agents to address this important need. On the Gram-positive side, the rapid increase in virulent community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infections as a cause of pneumonia emphasizes the importance of developing more agents that are active against MRSA and that are effective for treating pneumonia. Finally, the importance of indwelling devices as a nidus for nosocomial infections emphasizes the need for effective agents for treating biofilm-associated device infection both inside and outside of the hospital.

Bacterial infections remain major causes of morbidity and mortality in hospitals around the world. One recent study estimated that nosocomial *Staphylococcus aureus* infections alone resulted in 2.7 million days in excess length of stay, 9.5 billion dollars in extra hospital charges and nearly 12,000 inpatient deaths per year [1]. As *S. aureus* is estimated to be responsible for roughly 16% of nosocomial infections in the U.S., the tremendous toll of nosocomial bacterial infections in the United States is obvious. That these numbers exist despite the large number of antimicrobial agents that are available to practicing physicians indicates that some portion of the morbidity and mortality associated with bacterial infections may well be immune to the impact of current antimicrobial therapy. Factors such as the increasing age of the inpatient population, underlying diseases, advanced immunosuppression, immobility and lapses in appropriate infection control practices all contribute to infectious morbidity in ways that antibiotics may be able to impact only minimally. Moreover, current antimicrobial therapy is increasingly compromised by the emergence and spread of bacteria resistant to commonly used antimicrobial agents. This resistance is due largely to the substantial quantities of antibiotics that are administered in health care, and even non-health care settings. Empiric use of antimicrobial agents for questionable infections, spectra of therapy that are more broad than are indicated by likely pathogens, prolonged therapy after successful treatment and widespread use of antibiotics in food industries all contribute in significant ways to the growing problem of resistance. Some of the more problematic resistant bacteria that have emerged in recent years and that will be discussed in this paper are listed in Table 1.

Clearly, however, with such staggering numbers for morbidity and mortality there must be ways in which antibiotic therapy can be improved. In this paper, I will discuss the unmet needs in antibacterial therapy of human infections. In order to put these unmet needs in perspective, it

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Table 1 – Evolving and persistent unmet needs in antibacterial therapy

Evolving needs	Persistent need
Multi-resistant Gram-negative bacteria *Pseudomonas aeruginosa*, *Acinetobacter baumanni*, *Stenotrophomonas maltophilia*, Community-acquired (CA) UTI *Escherichia coli* CA- and hospital-acquired pneumonia *Staphylococcus aureus*	Antibiotics for biofilm-related infections *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus* spp., *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter* spp., *Pseudomonas aeruginosa*, *Acinetobacter baumanni*, *Stenotrophomonas maltophilia*

is worthwhile to spend a few moments discussing the evolution of antimicrobial therapy that has brought us to our current position.

The introduction of the first antibacterial compounds (streptomycin, sulfa, penicillin) met the major needs of the time by providing effective therapy for staphylococcal, streptococcal and mycobacterial infections. *S. aureus*, *Streptococcus pyogenes* and *pneumoniae* and *Mycobacterium tuberculosis* were major scourges that filled hospitals and sanitariums. The impacts of the first antibacterial compounds have been described as miraculous by many observers [2]. Soon, however, this impact was muted by the emergence of resistance, particularly in *S. aureus* and *M. tuberculosis*. The remarkable success of these agents against their primary targets also led to the emergence of previously ignored bacteria, primarily Gram-negative bacilli, as important pathogens. New classes of antibiotics, such as the cephalosporins, monobactams and carbapenems were developed over the years to address the emergence of progressively resistant Gram-negative bacilli. Similarly, semi-synthetic penicillins, vancomycin, macrolides and tetracyclines provided important and expanded coverage for many Gram-positive bacteria.

1. The emergence and spread of resistance in Gram-positive bacteria

By the mid- to late-1980s, there was a general sentiment (in all but the infectious diseases community) that the problem of antimicrobial resistance had been conquered and that the antibiotic market was saturated, leading to the first wave of pharmaceutical companies abandoning the field of antibacterial development. After all, we now had agents from several classes (penicillins, cephalosporins, monobactams, carbapenems, fluoroquinolones and aminoglycosides) that had clinically important activity against that most resistant and troublesome nosocomial pathogen, *Pseudomonas aeruginosa*. There were rumblings of increasing problems associated with methicillin resistance in staphylococci, but vancomycin remained universally active against virtually all clinically important Gram-positive pathogens, rendering concerns about *S. aureus* less urgent.

Then came the emergence and rapid spread of vancomycin resistance in ampicillin-resistant strains of *Enterococcus faecium* (VRE) [3]. Infections caused by these bacteria appeared virtually untreatable, and were afflicting increasing numbers of patients in intensive care units and transplant wards. Moreover, genetic studies had suggested that enterococci and staphylococci were able to exchange genetic materials [4], raising fears that the mobile enterococcal determinants would enter staphylococci and create untreatable staphylococcal infections. Several companies within the pharmaceutical industry responded by ramping up efforts to identify compounds that would be active against multi-resistant Gram-positive pathogens. Strategies included poring over old chemical libraries to identify compounds with the required activity, reviving antibiotics that had been shelved because of toxicities or other problems and investing heavily in genomics to try to identify new targets for inhibition.

These efforts have to a large extent paid off, yielding no fewer than four newly licensed agents in the past 5 years that have in vitro activity against both methicillin-resistant *S. aureus* (MRSA) and VRE. Three of these agents (quinupristin–dalfopristin, linezolid and daptomycin) have spectra that are restricted to Gram-positive bacteria, only one of which (linezolid) is available in oral form. The fourth compound, tigecycline, also has significant Gram-negative activity, but its spectrum does not include *P. aeruginosa*. So it is reasonable to say that the problem of antimicrobial therapy of infections caused by most resistant Gram-positive bacteria is in a far better state than it was a decade ago, and will improve even further as new agents currently being tested make their way to the market.

An issue of major and growing concern is the prevalence of methicillin-resistant MRSA in both the community and hospital settings [5,6]. In the hospital, data from the National Nosocomial Infection Surveillance indicate that nearly 60% of *S. aureus* strains are now resistant to methicillin, indicating resistance not only to all β-lactams, but also to most other commonly used antibiotics [7]. These strains are common causes of ventilator-associated pneumonia. In the community, aggressive strains of MRSA are now common causes of soft tissue infections, and are being increasingly recognized as causes of community-acquired pneumonia [8]. It is primarily in the area of pneumonia that the major danger exists. Recent data suggest that vancomycin, which does not achieve significant concentrations in the alveolar lining fluid, may be inferior to linezolid for treatment of MRSA pneumonia [9]. Daptomycin was found to be ineffective for treating pneumonia in its clinical trials. Quinupristin–dalfopristin is active against MRSA, but it does not have an FDA approved indication for treating pneumonia. Moreover, there is often a reluctance to use quinupristin–dalfopristin in many areas because of its high cost and the muscle aches that often accompany its administration. Linezolid is indicated for treatment of

pneumonia, and tigecycline clinical trials for pneumonia treatment are in progress. However, neither of these agents is bactericidal, so development of a bactericidal agent that would be effective for treating MRSA pneumonia would have great appeal. The development of cephalosporins with activity against MRSA will be followed with great interest, given the long and successful history of these agents in treating pneumonia [10].

This progress has occurred amidst a second exodus of pharmaceutical firms from the antibacterial field. Much of the exodus has been attributed to similar ''saturation'' arguments that were made in the past. While resistant bacteria are important, they represent a relatively small percentage overall of bacterial infections. For many resistant bacteria, we already have several agents with clinical efficacy. The relative rarity of multi-resistant infections, the almost invariable association of resistant infections with prior antimicrobial therapy and the need to perform clinical trials of new antibiotics on ''antibiotic-naïve'' patients makes enrolling patients with resistant infections difficult, and therefore more expensive. The advent of the ''blockbuster'' drug era has made shareholders less enamored of drugs that are unlikely to yield yearly revenues under one billion dollars. Finally, translating genomic information into reasonable targets, and then identifying inhibitors of the new targets that will be deliverable to the appropriate site within the bacterium has proved to be a more daunting challenge than first imagined.

2. The re-emergence of resistant Gram-negative bacteria

The shrinking pool of large pharma firms willing to invest in antibacterial research, along with the almost exclusive focus of those that remain on drugs with activity against resistant Gram-positive bacteria, has been associated with a dearth of research into antimicrobial agents with activity against resistant Gram-negative bacteria. Unfortunately, the selective pressure exerted by current antibiotics against Gram-negative bacteria has continued, resulting in a growing resistance problem in many hospitals in this country.

The problem of multi-resistant Gram-negative bacilli is indeed a daunting one for current pharmaceutical firms. One of the major problems is that the most problematic resistant bacteria are among the most naturally resistant organisms even in the absence of antimicrobial selective pressure. *P. aeruginosa*, *Acinetobacter baumanni* and *Stenotrophomonas maltophilia* are three important pathogens that are resistant to many commonly used antibiotics. Moreover, in the presence of selective pressure they can emerge resistant to even the relatively few antibiotics that have activity. It is worthwhile to consider the common characteristics of these bacteria.

P. aeruginosa, *A. baumanni* and *S. maltophilia* are all Gram-negative bacteria that are primarily important as causes of infections in immunocompromised patients, especially patients in intensive care units. Lower respiratory tract infections, wounds infections, urinary tract infections and bacteremias are common clinical syndromes associated with these species. Of the three, *P. aeruginosa* has been the most extensively studied. *P. aeruginosa* has many intrinsic characteristics that make it less susceptible to the activity of commonly used antimicrobial agents. The porins inserted into its outer membrane (the channels through which most antibiotics enter the periplasmic space) have a considerably slower transit times than do the porins of most other common Gram-negative pathogens [11]. This slow transit time is important since it can improve the odds that other defense mechanisms will be effective. *P. aeruginosa* is also able to decrease the quantities of specific porins in response to antimicrobial selective pressure. The best example of this is the reduction in quantities of the OprD2 porin in response to imipenem exposure [12]. Imipenem enters into the *P. aeruginosa* periplasmic space primarily through the OprD2 porin. As a zwitterion, imipenem traverses this porin quickly, offering it an advantage in overcoming the relatively weak (against imipenem) β-lactamase expressed from the *P. aeruginosa* chromosome. Prior studies have indicated that neither increased expression of the β-lactamase alone nor reduction of the OprD2 porin alone will yield imipenem resistance [12]. However, when both conditions are present, clinically important levels of resistance result. High levels of *P. aeruginosa* chromosomal β-lactamase are always present after exposure to imipenem, since that antibiotic is a very potent inducer of expression of that enzyme. Reductions of the OprD2 porin also appear easy for *P. aeruginosa* to achieve, since nearly 40% of patients treated with imipenem for a lower respiratory tract infection will yield a resistant strain before the end of therapy [13].

In addition to reduced access, *P. aeruginosa* also expresses a wide variety of modifying enzymes that reduce the activity of antibiotic once they have entered the cell. A large number of β-lactamases have been described in *P. aeruginosa*. The chromosomal cephalosporinase has been well described, and confers resistance to cephalosporins (with the possible exception of the fourth generation compounds cefepime and cefpirome), as well as penicillins, aztreonam and, under the proper circumstances (see above) imipenem. In addition, a variety of plasmid-mediated enzymes of different classes have been described that hydrolyze a broad spectrum of β-lactam agents. *P. aeruginosa* also expresses a variety of aminoglycoside-modifying enzymes, reducing the activities of that class of antibiotics.

Perhaps most important from the perspective of designing new drugs with activity against *P. aeruginosa* is the expression of multi-drug efflux pumps. The most important of these are the three component resistance-nodulation-cell-division (RND) pumps. Genomic analysis indicates that *P. aeruginosa* likely has 12 such pumps [14]. Seven have been characterized to date, with six of these confirmed to efflux antibiotics [15]. Only one (MexAB-OprM) is expressed under basal conditions, but other can be activated when the primary pump is not expressed, or when exposure to a specific antibiotic makes pump activation advantageous for the organism. The pumps serve two important roles in expression of resistance. The first is obviously to confer resistance by themselves, although the level of resistance conferred by most pumps alone is relatively modest [16]. The second is to provide an environment in which other resistance mechanisms confer a high enough level of resistance to allow selection in an antimicrobial-rich environment. This sort of collaboration is particularly important for

selection of fluoroquinolone resistance, in which significant levels of resistance often require two individual mutations in cellular topoisomerase genes. The combination of a single mutation (which by itself would not confer selectable levels of resistance) with a pump activation can yield selectable levels of resistance, allowing time for the second topoisomerase mutation to occur—yielding resistance even in the absence of pump activation.

Although less is known about *A. baumanni* and *S. maltophilia*, the above description of *P. aeruginosa* largely applies to them as well. Both have been shown to express a variety of modifying enzymes. *S. maltophilia* produces a metallo-β-lactamase that confers intrinsic resistance to carbapenems [17]. *S. maltophilia* only became a significant pathogen in intensive care units after imipenem was introduced as a clinical agent. *A. baumanni* expresses a variety of enzymes, including an intrinsic chromosomal cephalosporinase and a variety of plasmid-encoded acquired enzymes, including some metallo-enzymes that hydrolyze carbapenems [18]. Both *A. baumanni* and *S. maltophilia* have been shown to encode RND-type pumps [19,20], although the number of these pumps within the species and their specific activities are not as well characterized as those of *P. aeruginosa*.

The importance of RND-type efflux pumps cannot be understated as a challenge for developing new antimicrobials with activity against these pathogens. These are general pumps, designed to efflux a variety of materials that are toxic to the bacterial cell. This broad spectrum, combined with the number of different pumps encoded by the species, makes it conceivable, and perhaps even likely, that these species will be able to efflux virtually any compound that proves to be toxic. Target-based antimicrobial discovery programs therefore run the risk of large investments to identify a novel target and an effective inhibitor, only to find that the compound cannot achieve concentrations high enough at the target to effectively inhibit it.

Creating pump inhibitors has tremendous appeal. The ability to negate the activity of these efflux pumps could return activity not only to currently available antibiotics, but may preserve the activity of antibiotics to come [16]. There are significant challenges to this strategy, however. Selective pump inhibitors tend to yield mutants in which a second pump has assumed the duties of the inhibited one. Broad-spectrum pump inhibitors are more difficult to develop, and may run a greater risk of being toxic to human cells, since we use similar pumps to detoxify our own cells. Still, the potential yield from an effective pump inhibitor is so great that further investigations are clearly warranted.

Gram-negative bacteria are common causes of community-acquired infections as well. In particular, *E. coli* continues to predominate as a cause of urinary tract infections in community-dwelling females. Ampicillin resistance rates are above 30%, and in some geographic areas trimethoprim-sulfamethoxazole resistance levels exceed 20%, making these antimicrobial agents inefficient for empiric treatment of urinary tract infections [21]. Many physicians are therefore using fluoroquinolones as first line agents. While at the moment a fluoroquinolone is a reliable empirical choice, rising fluoroquinolone resistance rates throughout the world and in the U.S. suggest that this strategy may not be effective into the future. As such, there is a clear unmet need for additional classes of antimicrobial agents that will be effective for empiric treatment of community-acquired urinary tract infections.

3. Biofilm resistance

Resistance to antimicrobial activity comes in many forms. Even strains that may be highly susceptible to antibiotics when grown in planktonic cultures may be quite resistant when grown in a biofilm. Most bacteria, and virtually all of the problematic multi-resistant nosocomial strains, have been shown to produce biofilms under the appropriate circumstance. The development of these microbial communities is a result of remarkable intracellular communication. In Gram-negative bacteria, this communication occurs most commonly through the elaboration of homoserine lactones, whereas Gram-positive bacteria communicate through the use of small peptides. Originally thought of as a barrier to antibiotic entry, biofilms have more recently shown to be penetrated rather well by some antibiotics. The real problem with bacterial resistance in biofilms appears to be some change in the bacterial characteristics that make them less susceptible to antibiotics. The nature of these changes is an area of intense investigation. One recent paper analyzed a transposon mutant of *P. aeruginosa* that was able to form phenotypically normal biofilms, but that was susceptible to antimicrobial agents of different classes (aminoglycosides, chloramphenicol and quinolones) [22]. The mutation in this case was mapped to the *ndvB* gene, which is required for synthesis of periplasmic glucans. These periplasmic glucans were further shown to bind tobramycin, leading the authors to hypothesize that the products of *ndvB*, which is transcribed only in biofilm cells, bind and sequester tobramycin within the periplasmic space, thereby preventing movement to the target ribosome in the cellular cytoplasm. There was some strain variation in the impact of the *ndvB* mutation, suggesting that it is not the entire explanation of biofilm resistance.

Biofilms form when bacteria (not bacterial) colonize foreign material, such as intravascular or urinary catheters, orthopedic devices and other implantable material. Approximately one-half of the nosocomial infections occurring each year in the United States are associated with indwelling devices [23]. Therefore, biofilms are major players in the hospital setting. The lack of activity of most antibiotics against biofilm bacteria necessitates, in many cases, removal of the infected device to optimize the chances for successful therapy. This necessity adds dramatically to the morbidity and mortality associated with nosocomial infections every year. Development of antimicrobial agents that have activity against biofilm bacteria, and which prove to be effective in treating infections without device removal, would be a major advance in antimicrobial therapy.

4. Conclusions

We have many highly effective antimicrobial agents that allow us to treat the majority of bacterial infections that occur in the

community and in the nosocomial setting. However, the constant evolution of our microbial flora in response to antimicrobial selective pressure creates inevitable gaps in coverage. Current gaps and those to be anticipated in the near future include drugs to effectively treat multidrug-resistant Gram-negative pathogens, effective oral therapy for treatment of community-acquired urinary tract infections and drugs active against MRSA that will be bactericidal and effective in treating pneumonia. Another substantial but largely unaddressed area of need is the treatment of biofilm-related infections. Development of effective agents in these areas will serve an important medical need and should find large enough markets to be financially rewarding.

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REFERENCES

[1] Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Smulders M, et al. The burden of *Staphylococcus aureus* infections on hospitals in the United States: an analysis of the 2000 and 2001 nationwide inpatient sample database. Arch Intern Med 2005;165(15):1756–61.
[2] Thomas L. The youngest science: notes of medicine watcher. New York, NY: Viking Press, 1983.
[3] Rice LB. Emergence of vancomycin-resistant enterococci. Emerg Infect Dis 2001;7(2):183–7.
[4] Rice LB, Carias LL. Transfer of Tn5355, a composite, multiresistance element from *Enterococcus faecalis*. J Bacteriol 1998;180:714–21.
[5] Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol 2001;9(10):486–93.
[6] Mongkolrattanothai K, Boyle S, Kahana MD, Daum RS. Severe *Staphylococcus aureus* infections caused by clonally related community-acquired methicillin-susceptible and methicillin-resistant isolates. Clin Infect Dis 2003;37(8):1050–8.
[7] National Nosocomial Infections Surveillance (NNIS) System Report. Data summary from January 1992 through June 2004. Issued October 2004. Am J Infect Control 2004;32(8):470–485.
[8] Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, et al. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. Clin Infect Dis 2005;40(1):100–7.
[9] Wunderink RG, Rello J, Cammarata SK, Croos-Dabrera RV, Kollef MH. Linezolid vs vancomycin: analysis of two double-blind studies of patients with methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia. Chest 2003;124(5):1789–97.
[10] Chambers HF. Evaluation of ceftobiprole in a rabbit model of aortic valve endocarditis due to methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus*. Antimicrob Agents Chemother 2005;49(3):884–8.
[11] Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 2003;67(4):593–656.
[12] Livermore DM. Interplay of impermeability and chromosomal β-lactamase activity in imipenem-resistant *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1992;36:2046–8.
[13] Acar JF. Therapy for lower respiratory tract infections with imipenem/cilastatin: a review of worldwide experience. Rev Infect Dis 1985;7(Suppl. 3):S513–7.
[14] Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, et al. Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. Nature 2000;406(6799):959–64.
[15] Li Y, Mima T, Komori Y, Morita Y, Kuroda T, Mizushima T, et al. A new member of the tripartite multidrug efflux pumps, MexVW-OprM, in *Pseudomonas aeruginosa*. J Antimicrob Chemother 2003;52(4):572–5.
[16] Kriengkauykiat J, Porter E, Lomovskaya O, Wong-Beringer A. Use of an efflux pump inhibitor to determine the prevalence of efflux pump-mediated fluoroquinolone resistance and multidrug resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2005;49(2):565–70.
[17] Walsh TR, Hall L, Assinder SJ, Nichols WW, Cartwright SJ, MacGowan AP, et al. Sequence analysis of the Ll metallo-beta-lactamase from *Xanthomonas maltophilia*. Biochim Biophys Acta 1994;1218(2):199–201.
[18] Hujer KM, Hamza NS, Hujer AM, Perez F, Helfand MS, Bethel CR, et al. Identification of a new allelic variant of the *Acinetobacter baumannii* cephalosporinase ADC-7 beta-lactamase: defining a unique family of class C enzymes. Antimicrob Agents Chemother 2005;49(7):2941–8.
[19] Alonso A, Martinez JL. Cloning and characterization of SmeDEF, a novel multidrug efflux pump from *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 2000;44(11):3079–86.
[20] Magnet S, Courvalin P, Lambert T. Resistance-nodulation-cell division-type efflux pump involved in aminoglycoside resistance in *Acinetobacter baumannii* strain BM4454. Antimicrob Agents Chemother 2001;45(12):3375–80.
[21] Gupta K, Sahm DF, Mayfield D, Stamm WE. Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis. Clin Infect Dis 2001;33(1):89–94.
[22] Mah TF, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. Nature 2003;426(6964):306–10.
[23] Darouiche RO. Treatment of infections associated with surgical implants. N Engl J Med 2004;350:1422–9.