Emerging Gram-Negative Antibiotic Resistance: Daunting Challenges, Declining Sensitivities, and Dire Consequences

Robert E Siegel MD

Emerging antibiotic resistance has created a major public health dilemma, compounded by a dearth of new antibiotic options. Multidrug-resistant Gram-negative organisms have received less attention than Gram-positive threats, such as methicillin-resistant *Staphylococcus aureus*, but are just as menacing. Pathogens such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* employ a variety of resistance mechanisms and are associated with dangerous nosocomial outbreaks. In some cases these pathogens have expressed resistance to all clinically available compounds. The emergence of extended-spectrum β-lactamase-producing organisms in the community has raised alarm. Furthermore, the carbapenems, currently the most successful class of antibiotics, are showing signs of vulnerability. While the search for new antibiotic options continues, there is urgent need to employ strategies that will slow the development of resistance to the current armamentarium, such as avoiding prolonged antibiotic use or under-dosing, using pharmacokinetic and pharmacodynamic principles to choose dosing regimens, and encouraging early and aggressive empirical therapy, followed by de-escalation and narrowing the antimicrobial spectrum when culture results become available. *Key words: antibiotics, multidrug resistance, Gram-negative pathogens, Pseudomonas aeruginosa, Acinetobacter baumannii, extended-spectrum β lactamases, carbapenems, de-escalation.* [Respir Care 2008;53(4):471–479. © 2008 Daedalus Enterprises]

Introduction

Increasing rates of bacterial resistance among common pathogens are threatening the effectiveness of even the most potent antibiotics. While the spread of multidrug-resistant Gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus,* routinely capture headlines, Gram-negative pathogens attract less attention, although their emergence and spread are associated with serious public health concerns.[1,2] Many clinical laboratories, for example, do not screen for extended-spectrum β-lacta-

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mase (ESBL) producing Enterobacteriaceae, although they are increasingly found in the community and associated with treatment failure.[3] It is time to intensify attention to Gram-negative resistance.

The introduction of new antibiotics has not kept pace with the increasing rate of resistance, leaving clinicians with fewer treatment options. In the 1990s, when Gram-positive pathogens were largely responsible for antimicrobial resistance, antimicrobial agents such as linezolid (Zyvox) and quinupristin/dalfopristin (Synercid) were developed to treat them. Few new effective antibiotics were developed and approved for Gram-negative infections.[4] Meanwhile, resistance among Gram-negative pathogens has been on the rise, and established treatment protocols are frequently ineffective.[4,5] A recent analysis found that of 506 new drugs in development, only 5 were antibiotics.[2] The pharmaceutical pipeline for new antibiotics is drying up.[2] The alarming scenario of a pathogen resistant to all available antibiotic classes is among the most critical problems facing physicians today and a major global public health concern.

The Impact of Resistance

According to a 2007 report from the Centers for Disease Control and Prevention, an estimated 1.7 million health-care-associated infections occur in American hospitals each year. These infections are associated with 99,000 deaths.[6] This is a huge jump from previous decades. As recently as 1992, only 13,300 people died from hospital-acquired infections in the United States.[7]

Teaching hospitals and centers that treat critically ill patients are particularly vulnerable to high rates of bacterial resistance. Risk factors associated with increased resistance among patients in the intensive care unit (ICU) include long hospital stay, advanced age, use of invasive devices, immunosuppression, lack of hospital personnel adherence to infection-control principles, and previous antibiotic use.[1] Repeated courses of antimicrobial therapy are common in acutely ill, febrile patients, who frequently have endotracheal tubes, urinary catheters, and central venous catheters.[1] In combination with host factors, indwelling devices are routes for transmission and colonization of resistant infections.[8] However, 2 principal drivers of resistance appear to be inadequate (or inappropriate) empirical antibiotic therapy and prolonged antibiotic use.[1]

Lengthy or inappropriate antimicrobial therapy allows microbes to mutate into new forms that help them survive antibiotics and quickly become new, dominant strains.[7] In prolonged courses even effective antibiotics may permit the development of multidrug-resistant pathogens. In one study, pediatric patients were treated for various respiratory tract infections with either a standard 10-day course of amoxicillin or high-dose, short-course amoxicillin therapy. At the end of 28 days, the high-dose, short-course therapy group had lower rates of penicillin-resistant *Streptococcus pneumoniae* and lower risk of resistance to trimethoprim/sulfamethoxazole.[9] The study demonstrated that (1) bacterial mutants become dominant if pathogens are exposed to an antimicrobial agent for a long period, and (2) resistance genes travel together, spreading via conjugation or bacteriophages. These newly emergent resistant strains prey on the weakest patients, leaving hospitals with more severely ill patients, higher health care costs, and rising mortality rates.[7]

Resistance rates continue to rise yearly. In a review of nosocomial infections in 2003, compared to 1998–2002, *Pseudomonas aeruginosa* was 15% more resistant to imipenem, 20% more resistant to third-generation cephalosporins, and 9% more resistant to quinolones (Fig. 1).[10]

Emerging Resistance in Gram-Negative Pathogens

Clearly, more resilient and dangerous Gram-negative pathogens have established themselves in hospitals. *P. aeruginosa* and *Acinetobacter baumannii* have in some cases expressed resistance to *all* clinically available compounds.[4] Recently, colistin, an older polymyxin antibiotic with a reputation for nephrotoxicity and neurotoxicity, has emerged as a salvage therapy for nosocomial infections caused by multidrug-resistant pathogens in the ICU.[4,11] However, colistin-resistant strains have recently been reported. Eighteen specimens containing colistin-resistant *Klebsiella pneumoniae* were cultured from 13 ICU patients in 2004 and 2005. All those patients had long hospitalization and long duration of colistin therapy (median 27 d).[12]

P. aeruginosa

P. aeruginosa is a highly virulent pathogen and the source of multiple types of infections, including pneumonia, urinary tract infection, bacteremia, and wound infection. Hospital-acquired pneumonia due to *P. aeruginosa* is associated with high mortality. In a prospective study at 2 tertiary-care teaching hospitals, the 30-day mortality among 150 patients with hospital-acquired *P. aeruginosa* infections was 37%.[13] *P. aeruginosa* has properties that make it particularly problematic to hospitals, including inherent resistance to many drug classes, the ability to acquire resistance through mutation, an increasing incidence of local resistance, and frequent appearance in serious infections.[14] In fact, this pathogen has more capability for circumventing the activities of antimicrobials than does virtually any other microorganism.[4]

In most cases, infections due to *P. aeruginosa* occur in a nosocomial setting in patients with comorbid illness and compromise from catheters, tubes, and surgery. There have,

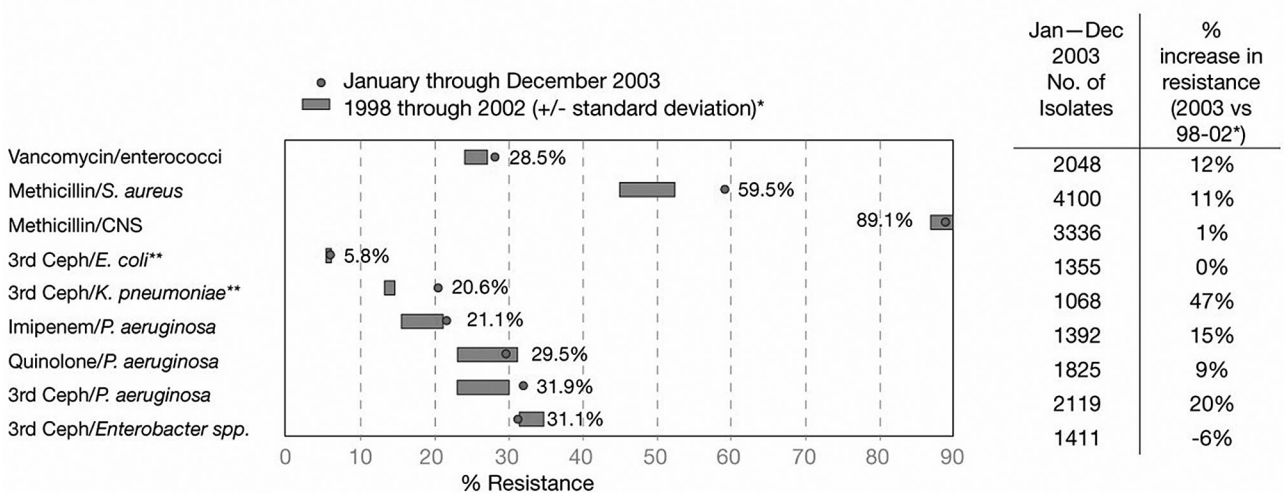

Fig. 1. Antibiotic-resistance rates among selected pathogens associated with nosocomial infections in intensive-care-unit patients, comparing the periods January through December 2003 to 1998 through 2002, using the National Nosocomial Infections Surveillance (NNIS) system. CNS = coagulase-negative staphylococci. 3rd Ceph = resistance to third-generation cephalosporins (ceftriaxone, cefotaxime, or ceftazidime). Quinolone = resistance to either ciprofloxacin or ofloxacin. * The percentage increase in resistance rate was calculated as (2003 rate – mean rate in 1998-2002)/mean rate in 1998-2002] × 100. ** "Resistance" of *Escherichia coli* or *Klebsiella pneumoniae* is the rate of nonsusceptibility in these organisms to either third-generation cephalosporins or aztreonam. (From Reference 10, with permission).

however, been increasing reports of *P. aeruginosa* lung infections in the community, usually in patients with structural lung disease or previous hospitalizations, but sometimes without clear predisposing factors.[15]

Several studies have found that multidrug-resistant strains of *P. aeruginosa* typically occur after prolonged exposure to antipseudomonal treatments or after empirical therapy.[13,15,16] A study of the incidence of *P. aeruginosa* resistance to β-lactam antibiotics in ICU patients found a high risk of emerging resistance during treatment with cefotaxime, imipenem, and piperacillin/tazobactam.[16] Reported high mortality, elevated minimum inhibitory concentration, and increased development of resistance to antimicrobial agents while on therapy have prompted guidelines to recommend treatment of *P. aeruginosa* with 2 pathogen-susceptible antibiotics, although there is limited evidence that combination therapy improves response to treatment.[17]

The antibiotic resistance of *P. aeruginosa* is compounded by its virulence, of which type III secretion is an important component. This complex system is used to translocate bacterial cytotoxins directly into host cells. These cytotoxins can inhibit phagocytosis and damage host tissues.[18,19] The ability of *P. aeruginosa* to form biofilms also increases its virulence. Bacteria within biofilms are often less susceptible to antibiotics. It is likely that most infections associated with foreign bodies (eg, ventilator-associated pneumonia [VAP], catheter-associated infections) involve biofilms.[4]

Virulence and resistance are intertwined in unique and complicated ways that can affect pathogenicity. Because biofilm-forming organisms are more resistant to antibacterial activity, antibiotics might select them, increasing the prevalence of chronic infections, or efflux pumps may extrude compounds involved in the host's defense, increasing a pathogen's virulence. On the other hand, strategies against virulence may lower resistance by reducing the number of pathogenic bacteria and the frequency of antibiotic exposure, thereby reducing mutations and the transfer of resistant genes between pathogens.[20]

A. baumannii

A. baumannii is an opportunistic Gram-negative pathogen that is difficult to treat, increasingly common in the ICU, and associated with nosocomial outbreaks worldwide.[5,21,22] Like *Pseudomonas,* it is intrinsically resistant to many antimicrobials.[5,21] Resistance of *Acinetobacter* isolates to amikacin, imipenem, and ceftazidime, among other antibiotics, is on the rise.[5]

A. baumannii frequently colonizes the ICU and can survive on wet or dry surfaces for prolonged periods.[23] The ability to thrive in the hospital environment contributes to its success: one investigation found viable *Acinetobacter* organisms on a bed rail 9 days after an infected patient was discharged.[23] In another study, computer keyboards in the ICU were identified as a reservoir.[24] One third of health care workers in a hospital ICU had *Acinetobacter* species cultured from their hands.[25] Similar to *Pseudomonas*, *A. baumannii* attacks patients with weakened defenses from illness or treatment, and those with invasive devices.[21] *A. baumannii* has been implicated in VAP, soft tissue in-

Table 1. Mechanisms of Antibiotic Resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

Mechanism	Means	Importance
Reduced access to target	Slow porin channels	High[16]
Increased antibiotic expulsion	Multiple drug-efflux pumps	Very high[31]
Inactivating enzymes	β-lactamases	Very high[31]
	Aminoglycoside-modifying enzymes	Very high[31]
Mutational resistance	Point mutations in topoisomerase genes	High in certain circumstances[4,31]
	Regulatory mutations that increase the expression of intrinsic genes and operons	High in certain circumstances[4,31]

fections, urinary tract infections, catheter-associated infections, and primary bacteremia.[4]

Extended-Spectrum β-Lactamase-Producing Organisms

Extended-spectrum β-lactamase-producing organisms (ESBLs) are plasmid-mediated enzymes that have mutated from more common β-lactamase enzymes. The presence of ESBL-producing pathogens is associated with higher morbidity and mortality than non-ESBL producers.[26] Concentrated use of third-generation cephalosporins is the most prominent risk factor for emergence of ESBL-producing pathogens. Other risk factors include prolonged antibiotic exposure, severe chronic illness, prior infections, prolonged hospital stay, residence in a long-term care facility, and an indwelling catheter.[26,27] Once an index case is identified, quick identification and isolation of an outbreak, with adherence to infection-control principles, is extremely important in preventing spread in the hospital environment.

ESBL-producing organisms were first detected in Europe and reported in the United States in 1988.[28,29] The prevalence of ESBL-producing Enterobacteriaceae ranges from 0% to 25%.[26] ESBLs have recently emerged in the community, raising further alarm. A surveillance study of ESBL-producing *Escherichia coli* infections in hospitals and the community in the period 2000 to 2002 was undertaken in Canada. The incidence was 5.5 cases/100,000 population per year. Seventy-one percent of the patients had community-onset disease.[30] Some researchers believe that the current situation regarding ESBL-producing pathogens mirrors the epidemiology of methicillin-resistant *S. aureus*, where community strains were quickly reported after their hospital presence was firmly established.[3]

Mechanisms of Antibiotic Resistance in Gram-Negative Pathogens

Problematic pathogens such as *P. aeruginosa* and *A. baumannii* thrive because they employ a variety of antibiotic resistance mechanisms (Table 1).[4] *P. aeruginosa* reduces an antibiotic's access to its target through the slowness of its outer membrane porin channels, which are 2 orders of magnitude slower at transporting solutes than are those of *E. coli.*[4,31] Impermeability, however, is a weaker resistance mechanism than efflux.[14] *P. aeruginosa* uses powerful efflux pumps to expel toxic compounds from both the cytoplasm and periplasm of the bacterial cell. At least 4 multidrug-efflux pump systems have been well characterized.[32]

P. aeruginosa also expresses an array of enzymes that inactivate antibiotics as they approach their targets.[4] In a classification known as the Ambler scheme, β-lactamases are divided into 4 major classes: A through D.[28] *P. aeruginosa* clinical isolates express all 4 Ambler classes, including metallo-enzymes (class B), which are active against the most stable of the β-lactam antibiotics, the carbapenems.[4]

The extraordinary cellular adaptability and survival of *P. aeruginosa,* honed over millennia, has now created states of *pan-resistance* at many medical centers.[33] Pan-resistance typically results from convergence of multiple resistance mechanisms.[33] A combination of up-regulated efflux, loss of OprD (a porin), and impermeability to aminoglycosides compromises every antibiotic class except the polymyxins.[14]

Because *A. baumannii* has become problematic relatively recently, less is known about its resistance mechanisms. Like *P. aeruginosa*, it expresses a variety of β-lactamases, including metallo-enzymes that can confer resistance to carbapenems. Multidrug efflux pumps have been described. *A. baumannii* also forms biofilms on endotracheal tubes and other invasive devices.[4]

ESBLs can hydrolyze β-lactam antibiotics. The plasmids responsible for ESBL production frequently carry genes that encode for various resistance mechanisms and multiple ESBL enzymes that target various antibiotic classes, which dramatically reduces antibiotic options.[26] Carbapenems, which are currently the treatment of choice for ESBLs, may be losing their effectiveness.[28] Studies have shown that a shift in empirical therapy to the carbapenems, due to the presence of ESBL producers, is associated with emerging resistance in *P. aeruginosa*, *A. baumannii*, and the ESBL-producing organisms themselves.[34,35]

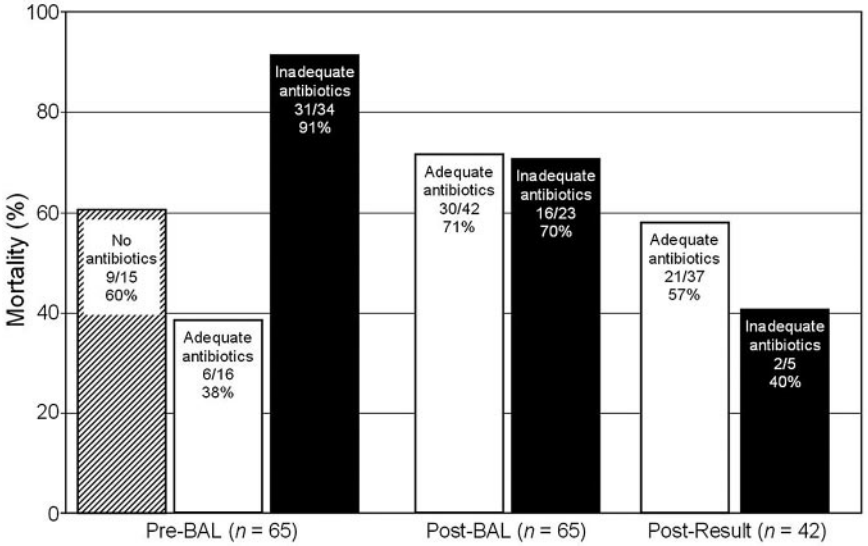

Fig. 2. Mortality rate versus adequacy of antibiotic therapy before bronchoalveolar lavage [pre-BAL], after BAL [post-BAL], and post-result [ie, evaluation of the therapy choices that were guided by the antimicrobial sensitivity data]). There was a statistically significant difference between adequate and inadequate therapy only at the pre-BAL time point, when mortality was lower in the patients who received adequate therapy. (Data from Reference 41.)

Carbapenemases are ESBL enzymes that hydrolyze or partially hydrolyze imipenem and/or meropenem. Because they often confer only partial resistance and are hard to detect, their presence may be underestimated.[36] The most clinically consequential are the Ambler class B metalloenzymes in *P. aeruginosa* and Enterobacteriaceae, and the Ambler class D oxacillinases in *A. baumannii*.[36,37] There have been recent reports in New York of carbapenemase-hydrolyzing β-lactamase variants of *K. pneumoniae* (*K. pneumoniae* carbapenemase or KPC) and *Enterobacter* species.[38–40] Among 257 isolates of *K. pneumoniae* in Brooklyn, New York, a disturbing 24% harbored the KPC-hydrolyzing β-lactamase.[39]

Resistance in Critical Hospital Infections

The Centers for Disease Control and Prevention reported that more than 70% of the bacteria that cause hospital-acquired infections are resistant to at least one of the antibiotics commonly selected to treat them.[7] Considerable evidence indicates that if initial treatment against an infecting microbe fails, the mortality rate is negatively affected, even if a switch to effective therapy occurs quickly.[41] In a study of patients with hospital-acquired pneumonia, a change from an inadequate to adequate antibiotic regimen after 2–3 days—when bronchoalveolar lavage (BAL) and antibiotic susceptibility results became available—did not improve the mortality rate, compared to patients consistently treated with inadequate therapy throughout their illness (Fig. 2).[41]

VAP typifies serious hospital-acquired infections made even more deadly in recent years by emerging resistance. In mechanically ventilated patients, VAP is clearly associated with higher morbidity, mortality, and health care costs.[8] The mortality rate associated with VAP is 20–50%, and there are reports of VAP mortality as high as 72%.[8,42] The patients with the highest mortality tend to be older, immunocompromised, have prolonged intubation, and are at greater risk of infection by *P. aeruginosa* and methicillin-resistant *S. aureus*.[42] Other pathogens associated with VAP include *Acinetobacter* species, *K. pneumoniae,* and *S. pneumoniae*.[8,43]

Infection Control

Contaminated respiratory therapy equipment and medical aerosols are a major source of VAP.[44] In a recent study, 28 episodes of pneumonia caused by *P. aeruginosa* were linked to contaminated bronchoscopes with defective biopsy port caps.[45] Frequent ventilator circuit changes do not prevent VAP and should be avoided, but condensate that has collected in the ventilator circuit requires special care. Critical care staff must guard against accidentally flushing condensate, which can become contaminated and enter the patient's airway or in-line nebulizer, at the bedside or during transport.[46]

Input from respiratory therapists and critical care staff is crucial to the prevention of infections such as VAP and the control of multidrug-resistant organisms. A protocol that includes rigorous disinfection of respiratory equipment and

bronchoscopes, and infection-control measures that avoid contamination of medical aerosols from nebulizers, will prevent this equipment from becoming reservoirs for resistant organisms.[44,46]

Correct diagnosis of VAP is the first step in infection control. Experts disagree on whether a clinical or bacteriologic approach is superior. Basing management decisions on clinical clues allows timely initiation of empirical therapy, but with this system some patients will be treated who do not need to be. Some investigators recommend culturing lower-respiratory-tract secretions via BAL or mini-BAL and waiting for results before beginning therapy.[47] Data suggest, however, that a delay in initiating appropriate broad-spectrum therapy is associated with higher mortality.[41,48]

The American Thoracic Society/Infectious Diseases Society of America guidelines[17] combine clinical and microbiologic approaches. They endorse early broad-spectrum treatment directed at likely pathogens, coupled with collection of a lower-respiratory-tract sample for culture and Gram stain, then de-escalation of antimicrobial therapy, if appropriate (Fig. 3).[17,46] Basic principles of de-escalation include stopping antibiotics when evidence of infection is lacking, and switching to an antibiotic that has a narrow antimicrobial spectrum when the pathogen is identified, which reduces the probability of emerging resistance.[17]

The prompt use of appropriate antibiotics for severe nosocomial pneumonia resulted in a 2-fold decrease in mortality.[42] However, selecting the initial therapy for VAP is not an easy task; local resistance patterns should be of paramount importance when initial antibiotic therapy is chosen. Even when resistance patterns and other issues are considered, successful management may prove elusive.

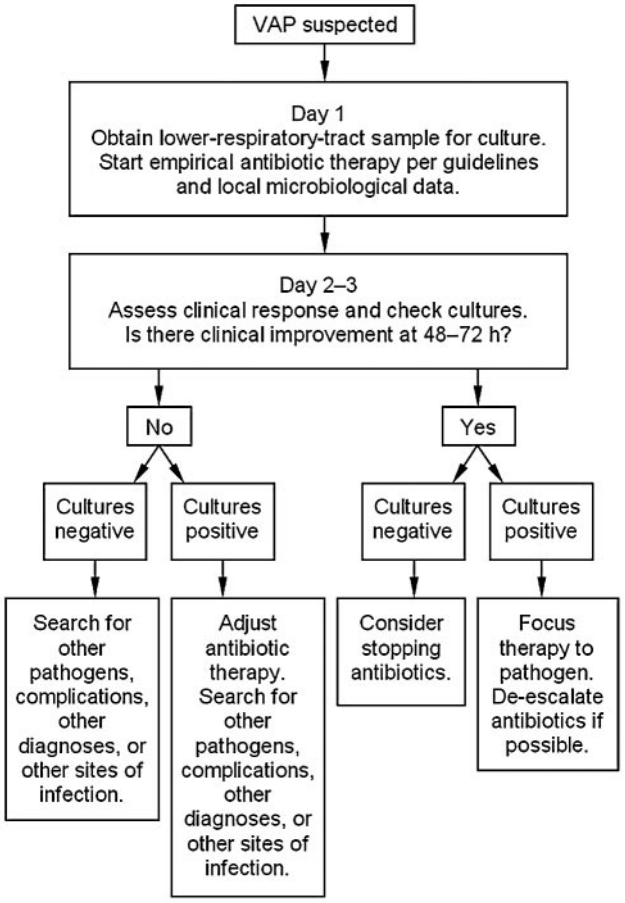

Fig. 3. Summary of management of ventilator-associated pneumonia (VAP). Empirical therapy is started as soon as there is clinical suspicion of pneumonia, based on existing guidelines and local microbiologic data. At the same time, a lower-respiratory-tract sample is collected. On days 2–3 the patient is re-evaluated based on clinical findings and culture results, and a decision is made whether to continue the current regimen, adjust, de-escalate, or stop therapy. (Adapted from References 17 and 46.)

Concerns About Carbapenems

Carbapenems have been the most successful class of antibiotics in evading emerging resistance. Imipenem and meropenem are considered to have the widest spectrum of any antimicrobial class, mainly because of their β-lactamase stability, but problems associated with their use are on the rise.[49,50] The biggest threat to carbapenems is the highly resistant Gram-negative pathogens represented by *P. aeruginosa* and *Acinetobacter species*.[49] The emergence of *K. pneumoniae* carbapenemases in *K. pneumoniae* and *Enterobacter* species in the northeastern United States is equally alarming.[38–40]

An important factor contributing to imipenem resistance is under-dosing—using a less-than-optimal dose to avoid potential central-nervous-system toxicity. Seizures have been seen in patients who received 4 g/d imipenem; when the dose was lowered to 2 g/d, seizure activity diminished.[51–53] Unfortunately, imipenem at 2 g/d may not achieve the minimum inhibitory concentration for *P. aeruginosa* for a sufficient time to ensure eradication.[54] β-lactam antibiotics accumulate in lung tissue at or just below serum levels.[55] The carbapenems and all β-lactam antibiotics kill on the basis of time-dependent, or concentration-independent, pharmacodynamics. The goal is to achieve a serum level above the minimum inhibitory concentration of the pathogenic bacteria for at least 40% of the dosing interval.[56] A subtherapeutic dose of an antibiotic can generate resistant organisms. In fact, when 2 g/d of imipenem was used instead of 4 g/d (because of reports of central-nervous-system toxicity with the 4-g dose), relapse and superinfections with *Pseudomonas* species were more common with imipenem (6 of 17 total episodes) than with ceftazidime (1 of 11 total episodes).[57]

The decrease in susceptibility to current antibiotics has made it difficult for today's clinicians to use antibiotics judiciously. One effective way to administer β-lactam an-

tibiotics is to infuse them over a prolonged period. This can safely keep the serum level sufficiently high for effective killing while limiting toxicity by minimizing the peak level. This approach has been tried with ceftazidime and with piperacillin/tazobactam.[58,59] Susceptibility testing has identified antimicrobial "break points" that predict an antibiotic's clinical success when surpassed, but may not prevent the development of resistance. Ongoing studies are determining "mutant-prevention concentrations" above which resistance is unlikely to occur.[60]

Summary

The history of infectious disease can be divided into 3 eras: the pre-antibiotic era, the antibiotic era, and the era of emerging infectious diseases.[49] The emerging resistance in today's world has created a major public health dilemma. The most potent antibiotic drug class currently available, the carbapenems, is forced to play a greater therapeutic role, but resistant strains employ mechanisms that can destroy the usefulness of this drug class. What can be done to slow the relentless progression of resistant pathogens?

Until the discovery and approval of new compounds, strategies can be employed to slow the development of resistance. For example, we must avoid under-dosing, which is a common yet often unrecognized factor associated with treatment failure and bacterial resistance.[1] An understanding of pharmacokinetic and pharmacodynamic principles can optimize antibiotic use, such as by increasing the time above the minimum inhibitory concentration with β-lactams, and by maximizing the peak level or area under the concentration curve with fluoroquinolones and aminoglycosides.[56]

Resistance containment depends on very early empirical and aggressive treatment for potentially resistant pathogens, followed by de-escalation and narrowing of the antimicrobial spectrum after identifying the pathogen. Empirical therapy should be discontinued altogether if a diagnosis of infection seems unlikely. De-escalation is a crucial infection-management technique and an effective strategy that balances the need to provide early adequate antibiotic therapy to high-risk patients and the objective of avoiding antibiotic overuse.[61]

Other strategies include prescribing drugs that have more than one mechanism of action or target, combining agents (where appropriate) to improve killing, and decreasing the duration of therapy.[1,49] Patients with VAP who received antimicrobial treatment for 8 days had no greater mortality or recurrent infections than did those who received 15 days of antibiotics. They did, however, have more antibiotic-free days.[62] Finally, adherence to infection-control principles by hospital personnel, which will often require further training and education, will create an improved best-practice environment for infection control. Only a continued commitment to these challenges and vigilance with respect to the use of antibiotics will allow advancement to the next era—one of renewed success against infectious disease.

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