Antimicrobial Resistance among and Therapeutic Options against Gram-Negative Pathogens

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Gram-negative bacterial pathogens are a common cause of infection, and the prevalence and rates of resistance among these pathogens to existing antimicrobial agents are increasing. β-Lactamase–mediated resistance is of particular concern. High-level resistance attributable to β-lactamase expression alone or in combination with other mechanisms is becoming increasingly prevalent among Enterobacteriaceae and gram-negative nonfermenting organisms. Doripenem is a new carbapenem with strong coverage of difficult-to-treat gram-negative bacterial pathogens. Its activity against *Pseudomonas aeruginosa* exceeds that of other carbapenems, and it has equivalent activity against *Acinetobacter* species and most Enterobacteriaceae. Thus, doripenem may be valuable alone or in combination with other agents in the treatment of serious gram-negative infections.

Gram-negative bacterial pathogens represent a major problem in hospital settings. In an analysis of surveillance data obtained from 1986 through 2003, the National Nosocomial Infection Surveillance System found that, each year, gram-negative pathogens were isolated in association with 65%–80% of all cases of intensive care unit (ICU)–acquired pneumonia, 40%–60% of all ICU-acquired surgical-site infections, ~70% of all ICU-acquired urinary tract infections, and ~25%–30% of all ICU-acquired bloodstream infections [1]. During the analysis period, the prevalence of *Acinetobacter* species increased among isolates associated with pneumonia, surgical-site infection, and urinary tract infection, as did the prevalence of *Klebsiella pneumoniae* among isolates associated with urinary tract infection, whereas the prevalence of *Escherichia coli* and *Enterobacter* species among isolates associated with surgical-site infection had decreased. Otherwise, there was little change associated with each type of ICU-acquired infection. In 2003, the final year of the analysis, the specific gram-negative pathogens most commonly associated with ICU-acquired infections were *Pseudomonas aeruginosa* in pneumonia, *K. pneumoniae* and *Enterobacter* species in bloodstream infection, *P. aeruginosa* and *Enterobacter* species in surgical-site infection, and *E. coli* and *P. aeruginosa* in urinary tract infection [1].

**THE GROWING PROBLEM OF ANTIMICROBIAL RESISTANCE AMONG GRAM-NEGATIVE PATHOGENS**

Data obtained by the National Nosocomial Infection Surveillance System indicate that, although gram-negative pathogens are commonly responsible for infection in the ICU, the prevalence of gram-negative pathogens as a cause of ICU-acquired infection has not increased substantially during the past 2 decades [1]. Nonetheless, the treatment of infections caused by gram-negative organisms has become considerably more challenging, primarily because of substantial increases in the frequency with which these organisms exhibit resistance to antimicrobial agents. In an examination of trends in susceptibility testing of bacterial isolates that were collected in association with ICU-acquired infections and were submitted to the National Nosocomial Infection Surveillance System by participating hospitals from 1986 through 2003 (mean total number of isolates submitted annually, 24,129), the prevalence of resistance to third-generation cephalosporins among *K. pneumoniae* isolates increased sharply, from <3% to >20%, from the late 1980s to the early 1990s, before it de-
creased slightly and then plateaued at ≈15%–20% for the remainder of the surveillance interval. Among *E. coli* isolates, the prevalence of resistance to this same class of antimicrobial agents increased more gradually but also more steadily during the surveillance period (figure 1A). The prevalence of resistance to third-generation cephalosporins increased among *P. aeruginosa* isolates from 1986 to 2003, as did the prevalence of resistance to imipenem (figure 1B). In addition, the percentage of *Acinetobacter* isolates exhibiting resistance to third-generation cephalosporins increased dramatically, from ≈25% in 1986 to almost 70% in 2003. Smaller but still substantial increases in resistance to amikacin and imipenem among *Acinetobacter* isolates were also reported (figure 1C) [1].

The therapeutic challenges that have arisen because of heightened antimicrobial resistance among gram-negative pathogens have been exacerbated by the stagnation in development of

![Figure 1](image_url)

novel antimicrobial agents to treat these pathogens, as was acknowledged in a 2006 report by the Infectious Diseases Society of America (IDSA) Antimicrobial Availability Task Force and an update of that report in 2009 [2]. In the 2009 update, 5 specific gram-negative organisms—*P. aeruginosa*, *Acinetobacter baumannii*, *K. pneumoniae*, *E. coli*, and *Enterobacter* species—were identified as problematic pathogens, because of clinical or public health concerns, associated infection with high attributable mortality rates, and unique virulence or drug-resistance factors.

Although available surveillance data provide concrete evidence of the growing problem of antimicrobial resistance among gram-negative organisms and the views of the IDSA Antimicrobial Availability Task Force underscore the seriousness of this problem, it is critical to note that resistance trends may vary greatly from one hospital to another within a limited geographic area, or even from one unit to another within an individual hospital. As a result, antimicrobial-susceptibility data from any given regional, national, or international surveillance study cannot reliably predict the drug-resistance profiles of pathogens isolated from an individual patient. The value of regional, national, and international surveillance data, therefore, lies not in the use of these data to guide treatment decisions on a case-by-case basis but, rather, in the illustration of large-scale antimicrobial-resistance trends that warrant attention [3].

**MECHANISMS OF ANTIMICROBIAL RESISTANCE IN GRAM-NEGATIVE PATHOGENS**

The genetic and biochemical mechanisms of antimicrobial resistance in gram-negative bacilli are numerous and diverse. For instance, resistance may arise through alterations in bacterial enzymes that are targeted by antimicrobial agents [4]. Of primary importance in this regard are alterations that may occur in penicillin-binding proteins (PBPs), a class of enzymes that catalyze bacterial cell wall synthesis. β-Lactam antibiotics exert their antimicrobial effects by binding antagonistically to PBPs, thereby compromising bacterial cell wall integrity. However, PBPs with a reduced affinity for β-lactams may emerge in gram-negative pathogens through the mutation of endogenous PBPs, and it is also possible for gram-negative pathogens to acquire low-affinity PBPs from related organisms via homologous recombination. Because the activity of β-lactam antibiotics depends on the ability of these agents to bind to PBPs, pathogenic organisms expressing reduced-affinity PBPs may be less susceptible to the effects of β-lactams. This is a frequent mechanism of resistance among gram-positive pathogens but occurs rarely among gram-negative bacteria.

Enzymes that mediate the destruction of antibiotic molecules contribute more frequently to resistance in gram-negative pathogens. Among the enzymes that warrant particular attention are β-lactamases, which catalyze the hydrolysis of β-lactam antibiotics, including AmpC β-lactamases, metallo-β-lactamases (MBLs), extended-spectrum β-lactamases (ESBLs), oxacillinases, and *K. pneumoniae* carbapenemases (KPCs) (table 1). With regard to specific modes of β-lactamase dissemination, the spread of AmpC β-lactamases among the Enterobacteriaceae has been linked to the plasmid-mediated transfer of genes that encode AmpC [5, 6]. Likewise, plasmid-mediated transfer appears to be the primary mechanism by which the transmission of KPCs among Enterobacteriaceae occurs [7]. Plasmid-mediated transfer has also been implicated in the propagation of MBLs, as has integron-mediated transfer, whereas the propagation of ESBLs has been attributed to multiple processes, including plasmid-mediated transfer, clonal expansion, and mutational events leading to the development of extended-spectrum activity in β-lactamases that had previously demonstrated narrow-spectrum activity [6].

Whereas ESBL- and KPC-mediated resistance may be found most commonly in Enterobacteriaceae, nonfermentative gram-negative bacteria may develop resistance more frequently to β-lactams via AmpC, MBL, oxacillinase enzymes, and non–lactamase-mediated pathways [6, 8, 9]. *P. aeruginosa* possesses an inducible, chromosomal AmpC β-lactamase that may, if derepressed because of mutational events, confer resistance to oxyiminocephalosporins [6]. Furthermore, *A. baumannii* possesses a noninducible, chromosomal AmpC β-lactamase that may, if overexpressed because of the insertion of a promoter sequence upstream of the *ampC* gene, produce an oxyiminocephalosporin-resistant phenotype [6, 10]. In addition, al-

### Table 1. Problematic β-lactamases found in Enterobacteriaceae and gram-negative nonfermenting organisms.

<table>
<thead>
<tr>
<th>Lactamase type</th>
<th>Associated resistance phenotype</th>
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<tbody>
<tr>
<td>AmpC β-lactamase</td>
<td>Resistance to all β-lactams except carbapenems^a</td>
</tr>
<tr>
<td>Metallo-β-lactamase</td>
<td>Resistance to all β-lactams except monobactams</td>
</tr>
<tr>
<td>Extended-spectrum β-lactamase</td>
<td>Resistance to oxyiminocephalosporins and monobactams</td>
</tr>
<tr>
<td>Oxacillinase</td>
<td>Resistance to carbapenems</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> carbapenemase</td>
<td>Resistance to all β-lactams</td>
</tr>
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^a Phenotype is observed only in those ampC-positive Enterobacteriaceae strains in which the ampC gene (and, thus, expression of the AmpC enzyme) is derepressed.
though the production of MBLs, ESBLs, or KPCs by gram-negative nonfermenting organisms is rare, *P. aeruginosa* isolates expressing such enzymes have been detected sporadically in Europe, Asia, and South America [9, 10]. The prevalence of MBL-expressing *P. aeruginosa* strains has increased steadily. The emergence of MBL-producing carbapenem-resistant *A. baumannii* strains has been reported as well. However, the oxacillinase carbapenemases—another group of β-lactamases—is the predominant family of enzymes associated with resistance to carbapenems in *A. baumannii* infection. *A. baumannii* strains exhibiting carbapenem-resistant phenotypes in association with oxacillinase carbapenemase production are now found worldwide, and outbreaks have occurred in Spain and Hong Kong [11].

Another common resistance pathway seen in gram-negative nonfermenting organisms involves down-regulation of the net intracellular intake of antibiotic molecules, either through up-regulation of endogenous efflux systems or through changes in cellular permeability to exogenous antibiotic molecules. In *P. aeruginosa*, up-regulation of the MexAB-OprM efflux system may result in reduced susceptibility to meropenem (but not imipenem) and in resistance to fluoroquinolones, penicillins, and cephalosporins. Up-regulation of efflux systems such as MexCD-OprJ, MexER-OprN, and MexXY-OprM may analogously give rise to various other resistance phenotypes [8, 9]. In *A. baumannii* infection, derepression of the AdeABC efflux system may result in resistance to aminoglycosides and reduced susceptibility to quinolones, tetracyclines, and trimethoprim, among other agents [9].

Furthermore, the permeability of *P. aeruginosa* strains to carbapenems may be lost through mutational events that suppress expression of OprD, a porin that forms a narrow transmembrane channel through which carbapenem molecules gain entry into pseudomonal cells, and this loss of permeability may result in reduced susceptibility to all carbapenems [8]. Such strains may remain susceptible to other β-lactam agents that penetrate via other porin proteins. Likewise, it has been shown that resistance to imipenem may arise secondarily to the loss of expression of the porin CaroO in *A. baumannii* [9]. Non–β-lactamase-mediated resistance mechanisms other than those involving modulation of antimicrobial influx or efflux may be operative in gram-negative pathogens [8, 9, 12]. For example, mutations in the GyrA subunit of DNA gyrase, the enzyme targeted by quinolone antibiotics, may confer decreased susceptibility to quinolones. Similarly, Enterobacteriaceae or non-fermenting organisms may express plasmid- or transposon-encoded aminoglycoside-modifying enzymes that can lead to reduced susceptibility to aminoglycoside antibiotics.

These multiple mechanisms of resistance in gram-negative pathogens occur frequently in the same strains, resulting in total resistance to all available antibiotics with the exception of polymyxins and tigecycline [13]. Such strains are found most often in *Klebsiella* species, *Pseudomonas* species, and *Acinetobacter* species, creating a major threat to successful therapy.

**TREATMENT OF INFECTIONS CAUSED BY GRAM-NEGATIVE PATHOGENS**

Infections due to gram-negative pathogens may occur in any human organ system, but they occur most frequently in the urinary, gastrointestinal, and respiratory tracts. Those that are mild and occur in the community (cystitis, pyelonephritis, cholecystitis, and sinusitis) are usually responsive to oral agents, such as fluoroquinolones, trimethoprim-sulfamethoxazole, cephalosporins, and ampicillin and its derivatives. Initial therapy should be chosen, in part, on the basis of local drug-susceptibility patterns. Culture samples should be obtained from the site of infection whenever possible, and therapy should be adjusted according to susceptibility testing results. More-serious gram-negative infections occur in patients in a hospital setting or in long-term care facilities. Among inpatients, the most problematic infections occur in ICUs. Patients in the ICU usually have serious comorbid conditions or have conditions compromised by invasive procedures, such as surgery, mechanical ventilation, and use of vascular or urinary catheters. These infections are often life-threatening and may be caused by gram-negative organisms that are resistant to multiple antibiotics. Several clinical studies from the past decade or earlier have provided convincing evidence that effective initial empirical antibiotic therapy, defined by ultimate drug-susceptibility results, improves survival. Such studies also indicate that later adjustment of inadequate initial therapy, after drug-susceptibility data become available, does not mitigate the adverse effect of inadequate initial therapy. Thus, an important therapeutic principle has emerged that supports aggressive, broad-spectrum initial empirical therapy of serious infections, followed by appropriate de-escalation of treatment according to the results of antibiotic-susceptibility data [14].

This principle is best implemented by reference to ongoing surveillance of antibiotic-susceptibility and -resistance data, which may be derived from surveillance studies of pathogenic organisms collected from international, national, regional, or local clinical sites. The most critical of these sources in the formulation of effective initial empirical therapy are local data regarding isolates recovered from the particular hospital, care unit, or specific patient [3]. Data from a specific patient may be obtained from earlier culture specimens taken from the infected patient that provide drug-susceptibility data regarding colonizing or previously infecting pathogens. The use of such information in the selection of empirical therapy for a particular patient is supported by studies that demonstrate that most invasive, multidrug-resistant, gram-negative infections are
caused by organisms that have colonized the patient before infection.

International, national, and regional surveillance of antibiotic-susceptibility profiles does not define appropriate empirical therapy for individual patients, but the profiles do inform clinicians of the potential need for antibiotics with activity against prevailing multidrug-resistant bacterial pathogens. It is clear from such surveillance studies that carbapenems are currently the agents most broadly active against gram-negative bacilli [15]. Among available carbapenems, doripenem has emerged as the agent in this group that is most active against P. aeruginosa, and its in vitro activity against Enterobacteriaceae is approximately equivalent to that of meropenem and imipenem [16]. In addition, its activity against Acinetobacter species is equivalent to that of imipenem. Doripenem’s greater in vitro activity against P. aeruginosa is consistent with findings indicating that mutational changes in OprD porin that result in decreased susceptibility are less likely to occur after exposure to doripenem than after exposure to other carbapenems [17].

**OPTIMIZING BACTERICIDAL ACTIVITY AGAINST GRAM-NEGATIVE PATHOGENS**

The pharmacodynamic characteristics of currently available antimicrobial agents play an important role in determining the dosing regimens that will provide optimal bactericidal exposure and that will minimize the development of drug resistance [18]. Pharmacodynamic studies using animal models of infection have shown that carbapenem antibiotics exhibit time-dependent bactericidal activity, producing maximal bactericidal effects when the free-drug concentration in plasma exceeds the minimum inhibitory concentration (MIC) of the infecting pathogen for at least 40% of the dosing interval [18]. The understanding of the pharmacodynamic properties of carbapenems has prompted the exploration of alternative dosing strategies, such as prolonged infusion, higher doses, and combination therapy, with the aim of designing a regimen that provides the highest probability of attaining the pharmacodynamic targets that produce maximal bactericidal exposure and that prevent the emergence of drug-resistant organisms [19–21].

In a study that used Monte Carlo simulation to determine pharmacodynamic target-attainment rates against specific pathogens (Enterobacteriaceae, P. aeruginosa, and Acinetobacter species), the meropenem infusion duration was extended to 3 h from the traditional 30 min [21]. The MICs and their frequencies were obtained from the Meropenem Yearly Susceptibility Test Information Collection, an ongoing, global hospital pathogen surveillance program. The study revealed that prolonging the infusion of meropenem (dosage, 1000 mg every 8 h) from 30 min to 3 h provided little benefit against Enterobacteriaceae, because of the low MICs for the susceptible isolates and the very high MICs for the resistant isolates. For P. aeruginosa and Acinetobacter species, pathogens with a greater percentage of isolates near the susceptibility breakpoint, a 3-h infusion of a higher meropenem dose (dosage, 2000 mg every 8 h) was needed to maintain a high probability of bactericidal exposure at MICs of 8 μg/mL or 16 μg/mL [21]. These findings suggest that, for serious infections potentially caused by P. aeruginosa or Acinetobacter species, a prolonged infusion of a higher meropenem dose is needed to provide adequate bactericidal exposure against intermediately resistant isolates. Several small clinical studies have evaluated meropenem administered by a 3-h infusion in hospitalized patients with serious infections [22, 23]. In a study conducted among 9 patients with ventilator-associated pneumonia, an empirical treatment regimen of meropenem at a dosage of 2000 mg every 8 h by 3-h infusion was found to provide meropenem serum concentrations above the MIC of 16 μg/mL for almost 60% of the 8-h dosing interval, providing bactericidal exposure for pathogens with higher MICs [22].

The findings from the pharmacodynamic studies demonstrating that prolonged infusions of meropenem provided a high probability of bactericidal exposure against pathogens with a higher MIC prompted the exploration of the potential benefits of prolonged infusion as a dosing strategy for doripenem. In a large, prospective, multicenter, randomized, open-label study involving patients in the ICU with ventilator-associated pneumonia, the clinical cure rate with doripenem treatment (500 mg every 8 h by 4-h infusion) was compared with that of imipenem treatment (500 mg every 6 h by 30-min infusion or 1000 mg every 8 h by 60-min infusion) [24]. The results demonstrated that doripenem was noninferior to imipenem. Among patients infected with P. aeruginosa, clinical cure occurred in 16 (80%) of 20 patients who received doripenem and 6 (43%) of 14 patients who received imipenem; the difference was not statistically significant. Among these same patients, microbiological cure with doripenem treatment (13 [65%] of 20 patients) was similarly greater than that with imipenem treatment (5 [36%] of 14 patients); again, this result was not statistically significant. Another study involving patients with complicated intra-abdominal infection showed that intravenous doripenem treatment at a dosage of 500 mg by 60-min infusion every 8 h was noninferior to meropenem treatment at a dosage of 1 g by 3–5 min infusion every 8 h [25]. These results suggest strongly that, for serious infections, doripenem, at a significantly lower dose, may provide clinical efficacy equivalent to that of other carbapenems, particularly when doripenem is administered in a more prolonged intravenous infusion. Whether this advantage can be leveraged by using larger doses of doripenem at prolonged infusion times to treat less-susceptible or carbapenem-resistant gram-negative infections remains to be determined. Doripenem is currently approved for the
treatment of complicated intra-abdominal and urinary tract infections caused by susceptible strains, including pyelonephritis and cases with concurrent bacteremia.

Combination antibiotic therapy represents a potentially valuable option for the treatment of infections caused by multidrug-resistant gram-negative organisms, particularly Klebsiella species, P. aeruginosa, and A. baumannii. Numerous studies have demonstrated the in vitro efficacy of combination therapy against multidrug-resistant pathogens that are not susceptible to any of the individual agents [26–28]. For example, in an investigation of 8 A. baumannii isolates that were resistant to imipenem alone, Yoon et al. [26] found that the combination of imipenem with subinhibitory concentrations of polymyxin B had bactericidal effects on 7 of the 8 isolates under study, whereas the combination of imipenem, polymyxin B, and rifampin had bactericidal effects on all 8 isolates within a 24-h period. Furthermore, even in certain situations in which the infecting organism is susceptible to monotherapy with a given antimicrobial agent, combination therapy may be more effective. In a randomized evaluation of 121 patients with bacteremia caused by P. aeruginosa susceptible to β-lactams and aminoglycosides, the addition of rifampin to a standard treatment regimen of a β-lactam antibiotic and an aminoglycoside was associated with an improved bacteriological cure rate [29].

The potential benefits of combination therapy in the treatment of infection caused by a highly drug-resistant pathogen may depend on the prevalent mechanisms of resistance. For instance, combination regimens that involve a carbapenem and a polymyxin antibiotic may be effective against pathogens that have acquired carbapenem resistance through the loss of cell membrane permeability, because polymyxins increase membrane permeability and may allow carbapenem penetration. The clinical utility of treatment regimens containing a carbapenem and a polymyxin, however, has yet to be confirmed in controlled clinical studies. In contrast, the concomitant administration of a polymyxin may not enhance the activity of a carbapenem antibiotic against a pathogen that exhibits carbapenem resistance due to carbapenemase production [26, 30]. Antibiotic dosing may influence the usefulness of combination therapy. Suboptimal dosing of antimicrobial agents may contribute to the emergence of antimicrobial-resistant pathogens. Thus, antimicrobial agents should be administered at the same doses in combination therapy as in monotherapy regimens [14].

CONCLUSION

Gram-negative pathogens are a common cause of infection in both community and hospital settings. A substantial proportion of hospital-associated pathogens exhibit resistance to common antimicrobial agents. Furthermore, the prevalence of antimicrobial resistance among gram-negative organisms appears to be increasing, with highly troubling forms of resistance developing in a growing number of organisms. In particular, highly drug-resistant phenotypes mediated by AmpC β-lactamases, MBLs, ESBLs, oxacillinases, and KPC enzymes have become increasingly prevalent among Enterobacteriaceae and nonfermenting gram-negative bacilli. To optimize clinical outcomes in the treatment of serious infections, prompt and appropriate empirical antimicrobial therapy is necessary. Carbapenem antibiotics remain active against most Enterobacteriaceae and also against a substantial percentage of P. aeruginosa strains. Of the commercially available carbapenems, doripenem, with its higher activity against P. aeruginosa (MIC, ≤2 mg/L against susceptible strains), may provide an advantage when used alone or in combination with other agents against organisms that are less susceptible or that are resistant to multiple conventional antibiotics.

References