The Current State of Multidrug-Resistant Gram-Negative Bacilli in North America

Insights from the Society of Infectious Diseases Pharmacists

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Although much of today’s media focuses on multidrug-resistant gram-positive bacteria such as methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus, resistance within gram-negative bacilli continues to rise, occasionally creating situations in which few or no antibiotics that retain activity are available. Extended-spectrum β-lactamase (ESBL)-(335,319),(409,407)(500,319),(573,407)(743,319),(816,407)(288,319),(361,407)-producing Escherichia coli and Klebsiella sp are emerging threats nationally. Although carbapenems are considered the antibiotic class of choice to treat ESBL-producing Enterobacteriaceae, the ability of these organisms to produce carbapenemases has now become apparent in some regions throughout the United States. Although still rare, Klebsiella sp that produce KPC-2 retain susceptibility only to tigecycline, polymyxins, and occasionally aminoglycosides. Multidrug resistance among Pseudomonas aeruginosa and Acinetobacter sp has always been apparent across many hospitals in the United States. Recent surveillance indicates increasing resistance to all currently available antibiotics, including carbapenems, cephalosporins, penicillins, fluoroquinolones, and aminoglycosides. Against many strains, only polymyxins retain activity; however, resistance has also been reported to these agents. Fortunately, resistance mechanisms such as metallo-β-lactamases are still rare in the United States. As no new antibiotics with novel mechanisms against many of these gram-negative bacilli are expected to be developed in the foreseeable future, careful and conservative use of agents combined with good infection control practices is required.

Key Words: gram-negative bacilli, multidrug resistance, treatment options.

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OUTLINE

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Bacterial resistance is an ever-evolving survival tactic that has enabled bacteria to outlast available antibiotics. Even in the early days of antibiotics, resistance within Staphylococcus aureus was documented soon after the introduction of penicillin (i.e., penicillinase-producing S.
aureus) and methicillin (i.e., methicillin-resistant S. aureus [MRSA]).

Although multidrug resistance among gram-positive organisms, such as MRSA and vancomycin-resistant Enterococcus, has stolen much of the media attention and caused the pharmaceutical industry to focus efforts on developing novel antibiotics to combat these organisms, perhaps a more concerning event has been developing simultaneously—that is, multidrug resistance among gram-negative bacilli. Gram-negative organisms still account for most of the nosocomial infections, including pneumonia, skin infections, intraabdominal sepsis, and urosepsis, and are reemerging as a significant cause of bloodstream infections.

Resistance among gram-negative bacilli is not necessarily a new phenomenon. The first report of enzymes in Escherichia coli that were able to inactivate penicillin was presented early after the introduction of the drug in the 1940s. Organisms such as Pseudomonas aeruginosa have always had a unique ability to evade new antimicrobial therapies and develop resistance; however, where resistance did develop, usually at least a few other therapeutic options were available for treatment. More recently, panresistant gram-negative bacilli have been documented at hospitals globally, and although still infrequent, such strains are beginning to emerge in the United States. Besides the glycyicyclines, there is notable concern with the limited amount of new antimicrobial classes developed in the last 20 years. As noted by the Infectious Diseases Society of America’s “Bad Bugs, No Drugs” campaign, during the next decade, no new antimicrobial classes are expected to be developed to target some of these multidrug-resistant gram-negative bacilli.

In addition to this unfortunate situation, concern revolves around the types of gram-negative bacilli that have obtained or developed this resistance. For instance, nonfermenting gram-negative bacteria such as P. aeruginosa and Acinetobacter baumannii are now multidrug resistant, which is defined as resistant to three or more of the following antibiotic classes: β-lactams, including penicillins, cephalosporins, and monobactams; carbapenems; fluoroquinolones; and aminoglycosides. Even more concerning, these bacteria can be panresistant, defined as resistant to all available antibiotic options. Meanwhile, once easily treatable organisms such as E. coli and Klebsiella sp now harbor resistance mechanisms, which make them nearly panresistant. Increased patient mortality, prolonged length of hospitalization, and greater hospital costs have resulted.

In the United States, health care costs associated with multidrug resistance in general have been estimated at several billion dollars.

Common Mechanisms of Resistance within Gram-Negative Bacilli

Gram-negative bacilli can develop resistance to
most antibiotics through four general methods: production of enzymes that destroy the integrity of the antibiotic; mutations at the binding site, thereby preventing some antibiotics from binding tightly; downregulation of outer membrane proteins, thus preventing the antibiotic from getting into the periplasmic space; and efflux pumps that efficiently remove an antibiotic from the cell. Often for multidrug resistance to occur, several mechanisms of resistance are contained in the same strain.

The production of β-lactamases is among the most common and clinically significant resistance mechanisms displayed by all gram-negative bacilli. β-Lactamases are enzymes that hydrolyze the β-lactam chemical structure and inactivate the drug. They are typically classified by either the Ambler classification or the Bush-Jacoby-Medeiros classification (Table 1). With the Ambler classification, β-lactamases are divided into four classes based on amino acid similarities. For example, classes A, C, and D are serine β-lactamases, whereas class β enzymes are zinc β-lactamases. The Bush-Jacoby-Medeiros classification divides β-lactamases into four groups and multiple subgroups, based on substrate and inhibitor profiles. Specific types of β-lactamases produced by multidrug-resistant gram-negative bacilli will be discussed later.

Gram-negative bacilli can also produce enzymes that destroy aminoglycosides (i.e., aminoglycoside-modifying enzymes); these can be acetyltransferases, adenyltransferases, or phosphoryltransferases. These enzymes chemically modify the aminoglycoside structure, thereby interfering with drug transport or preventing the antibiotic from binding to the 30S ribosomal subunit. Aminoglycoside-modifying enzymes are usually not solely responsible for aminoglycoside resistance in gram-negative bacilli, as impermeability, efflux, and novel aminoglycoside resistance gene cassettes are also to blame.

Other ways in which bacteria possess resistance is through reduced permeability and efflux pumps. Before binding to their target site, antibiotics must permeate through the outer membrane of the organisms through an outer membrane protein or porin. For example, loss of the 54-kD outer membrane protein, known as OprD, is the most common mechanism of imipenem resistance in P. aeruginosa. Often, reduced permeability works in conjunction with efflux pumps to reduce antibiotic concentrations and prevent accumulation in the bacteria. In P. aeruginosa, efflux pumps are most notably a three-component protein system located on the cytoplasmic membrane and the outer membrane porin. Resistance as a result of efflux pumps and/or porin changes is commonly associated with this pathogen. Similar to the β-lactamases, other gram-negative bacilli are also in possession of these resistance mechanisms but vary in the characteristics of the component protein system.

Finally, binding-site mutations are the most common resistance mechanisms for gram-negative bacilli against the fluoroquinolones. Along with efflux, mutations of the target enzymes topoisomerase II and IV result in significant increases in fluoroquinolone minimum inhibitory concentrations (MICs). These enzymes are encoded by gyrA and parC, respectively, and among gram-negative organisms, it appears that mutations in gyrA are the most important, occurring before parC mutations.

Extended-Spectrum β-Lactamase–Producing Gram-Negative Bacilli

Extended-spectrum β-lactamases (ESBLs) are often found in the Enterobacteriaceae family of gram-negative bacilli, particularly Klebsiella sp, E. coli, and Proteus mirabilis; however, other gram-negative bacilli have been documented to produce such enzymes, including Enterobacter sp, P. aeruginosa, Citrobacter freundii, Morganella morganii, and Serratia marcescens. Since the first description of an ESBL in 1983, more than 200 different enzymes are now characterized. (An up-to-date detailed summary of these enzymes can be found at www.lahey.org/studies/webt.htm.) Of the many families of ESBLs, nearly all belong to the Bush-Jacoby-Medeiros group 2be and include TEM, SHV, and CTX-M-type enzymes. Although the OXA-type β-lactamases (group 2d) in general do not hydrolyze the extended-spectrum cephalosporins significantly, certain OXA enzymes (e.g., OXA-10) do have weak activity and are therefore classified by some as ESBLs.

The ESBL-producing organisms demonstrate higher MICs to third-generation cephalosporins (i.e., cefotaxime, ceftazidime, ceftriaxone) but may not raise the MIC enough to define the organism as resistant to these antibiotics. As a result, clinical microbiology laboratories must first screen for isolates that could be an ESBL (e.g., ceftazidime MIC ≥ 2 µg/ml) and then confirm the presence of an ESBL by determining the change in MIC when clavulanic acid is added.
Once confirmed, all cephalosporins (except the cephemycins [cefoxitin and cefotetan]), penicillins (except piperacillin-tazobactam), and aztreonam should be reported as resistant. Although β-lactamases, by definition, can only inactivate β-lactam antibiotics, many ESBL-producing bacteria also display resistance to trimethoprim-sulfamethoxazole, fluoroquinolones, and aminoglycosides, as their respective resistance mechanisms can be carried on the genes encoding for ESBLs on the bacterial plasmid. In contrast, the carbapenem class of antibiotics has greater stability against group 2β enzymes and is therefore considered by most authorities to be the antibiotic class of choice for the treatment of severe infections caused by an ESBL-producing organism. It is also important to note that many ESBL-producing organisms may produce other degrading enzymes, such as an AmpC cephalosporinase. In this case, cefoxitin, cefotetan, and potentially piperacillin-tazobactam resistance would also be apparent.

The prevalence of ESBL production varies significantly depending on geographic region, general setting (hospital or community), institution, and even hospital unit. Generally, ESBL-producing Enterobacteriaceae are more often found outside the United States, with parts of Latin America and Europe having rates as high as 60%. In the United States, rates of ESBL-producing *Klebsiella pneumoniae* and *E. coli* have been approximately 10% and 5%, respectively, with typically higher rates of ESBL-producing *K. pneumoniae* in the mid-Atlantic region (~20%). Variations also can exist within hospitals of a region. For instance, a Brooklyn, New York, hospital reported that 34% of their *K. pneumoniae* produced ESBLs, whereas a hospital in Hartford, Connecticut, reported ESBLs at a rate of 9.6% for *Klebsiella* sp. A more recent national surveillance study showed the number of confirmed ESBL *Klebsiella* sp and *E. coli* at 10.9% and 3.1%, respectively. Table 2 shows current susceptibility rates for several antibiotics against *K. pneumoniae*, as defined by the presence of an ESBL. Only imipenem, tigecycline, and amikacin retained high susceptibilities against the ESBL-producing organisms in this data set. The lower susceptibility rate reported for imipenem against *K. pneumoniae* likely was a result of KPC-producing organisms (discussed below).

### Carbapenemase-Producing *Klebsiella pneumoniae*

*Klebsiella* sp that produce a carbapenemase known as KPC are a serious concern because of high-level resistance against most antibiotics. The KPC enzymes are a class A, group 2β β-lactamase that efficiently hydrolyze carbapenems, as well as other β-lactam antibiotics. The first *K. pneumoniae* to produce a KPC (duly named KPC-1) was isolated in North Carolina during the Project Intensive Care Antimicrobial Resistance Epidemiology (ICARE) surveillance study in the late 1990s. However, an outbreak of KPC-producing *Klebsiella* sp in Brooklyn, New York, provides most of the epidemiology data we have for organisms producing this enzyme. The New York KPC is genetically distinct from KPC-1 and, therefore, is referred to as KPC-2. In two reports of New York hospitals, KPC-2 has been implicated in 15% of ESBL-possessing *K. pneumoniae* and 24% of total *K. pneumoniae* infections. The emergence of KPC-2 has also surfaced in other parts of the U.S. mid-Atlantic region; however, these enzymes are still rare in the United States. Areas outside the United States have also reported KPC-2 carbapenem resistance. In Israel and Colombia, KPC-2-type resistance was discovered in one patient with *E. coli* and two patients with *K. pneumoniae*. More alarming is the recent report of KPC-2 spreading to seven *E. coli* isolates among three New York City hospitals. In addition, KPC-3, -4, and -5 enzymes are now reported in other *E. coli* and *Enterobacter* sp strains outside of New York City (Stephen Jenkins, Ph.D., personal communication, April 15, 2007). Organisms that produce KPC have similar resistance profiles to

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Total Isolates (n=543)</th>
<th>ESBL-Producing Isolates (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>95.2</td>
<td>72.1</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>3.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Piperacillin-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tazobactam</td>
<td>88.6</td>
<td>49.2</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>84.3</td>
<td>6.6</td>
</tr>
<tr>
<td>Cefepime</td>
<td>94.9</td>
<td>62.3</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>88.2</td>
<td>24.6</td>
</tr>
<tr>
<td>Amikacin</td>
<td>97.4</td>
<td>88.5</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>83.3</td>
<td>23.0</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>93.7</td>
<td>93.4</td>
</tr>
<tr>
<td>Minocycline</td>
<td>83.1</td>
<td>75.4</td>
</tr>
</tbody>
</table>

ESBL = extended-spectrum β-lactamase. Data were collected during the 2004 Tigecycline Evaluation and Surveillance Trial (TEST program) in the United States. Susceptibility defined by the Clinical and Laboratory Standards Institute.
most ESBLs, but with the addition of carbapenem resistance. As such, only tigecycline, polymyxins, other tetracyclines (at times), and aminoglycosides (at times) retain activity. An improved method of detecting these organisms in the clinical microbiology laboratory is required since many of these organisms can also coproduce ESBLs and be otherwise reported as such.

**Pseudomonas aeruginosa**

*Pseudomonas aeruginosa* is a ubiquitous, non-fermenting, gram-negative, opportunistic pathogen that has the tendency to infect patients who are immunocompromised, critically ill, burned, and/or diabetic. Although rare, otherwise healthy patients can acquire skin infections from this pathogen through skin punctures of the lower extremity or from hot tubs.⁴⁶ In the hospital, however, *P. aeruginosa* is the second most common pathogen, particularly in those with lower respiratory tract infections.⁴⁷ When patients with cystic fibrosis acquire *P. aeruginosa*, it is believed to be near impossible to eradicate the pathogen.⁴⁸,⁴⁹ The presence of a biofilm allows *P. aeruginosa* to adhere to different medical devices and the airways of patients with cystic fibrosis. The idea of eradication through intense antimicrobial therapy may lead to significant selection of *P. aeruginosa* resistance.

In general, *P. aeruginosa* has a strong tendency to become a multidrug-resistant pathogen. Usually this process occurs as a result of both cross-infection with resistant strains and acquisition of drug resistance during treatment.⁵⁰,⁵¹ A steady rise in the pattern of multidrug resistance (defined in various studies as resistance to three or more antipseudomonal classes) in patients in the intensive care unit (ICU) and those not in an ICU has been observed.⁵² Although these resistance trends were believed to be at a plateau, recent 2005 surveillance data indicate that resistance rates for all drugs tested were greater when compared with 2004 or 2000 data.⁵³–⁵⁵ Table 3 displays current susceptibility rates for common antipseudomonal antibiotics in the United States as reported by the 2005 Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) study.⁵⁸ Data were collected during the 2005 Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) surveillance study in the United States.⁵⁸

<table>
<thead>
<tr>
<th>Antibiotic (%)</th>
<th>P. aeruginosa (n=589)</th>
<th>Acinetobacter sp (n=125)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam</td>
<td>74.2</td>
<td>64.0</td>
</tr>
<tr>
<td>Cefepime</td>
<td>86.9</td>
<td>60.8</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>86.9</td>
<td>60.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>72.5</td>
<td>72.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>83.9</td>
<td>92.0</td>
</tr>
<tr>
<td>Imipenem</td>
<td>84.4</td>
<td>62.0</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>69.4</td>
<td>85.6</td>
</tr>
<tr>
<td>Meropenem</td>
<td>87.6</td>
<td>59.2</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>91.0</td>
<td>92.0</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>88.6</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Susceptibility Rates for Selected Antibiotics Against *Pseudomonas aeruginosa* and *Acinetobacter* Species

Data were collected during the 2005 Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) surveillance study in the United States.⁵⁸

*Susceptibility defined by the Clinical and Laboratory Standards Institute.*

Although once widely used for infections caused by *P. aeruginosa*, the fluoroquinolone class of antibiotics has fallen out of favor due to rapidly emerging resistance among this organism. One group of authors assessed national antimicrobial resistance rates against 35,790 nonduplicate gram-negative rods recovered from patients in an ICU between 1994 and 2000.⁵⁷ Of these, 8244 (23%) were *P. aeruginosa*, and overall ciprofloxacin susceptibility was 76%. More alarming was the correlation between increasing fluoroquinolone use from year to year and the increase in resistance. Resistance increased from 16% in 1994 to over 30% in 2000; furthermore, ciprofloxacin resistant isolates were more likely to also be resistant to gentamicin, ceftazidime, imipenem, and amikacin.

Among the many types of resistance mechanisms that *P. aeruginosa* possesses, those that cause resistance to carbapenem antibiotics might be the most concerning, since there are few options available for *P. aeruginosa* that are carbapenem resistant. As previously mentioned, imipenem resistance within *P. aeruginosa* is most commonly associated with the loss of the OprD porin.⁵⁶
Mutational loss of OprD is frequent during imipenem therapy, with resistance emerging in as high as 25% of patients receiving the antibiotic.\textsuperscript{38} In contrast, loss of this porin increases the meropenem MIC but does not, by itself, cause clinical resistance. Clinically significant resistance to meropenem requires both loss of OprD and upregulation of the MexAB-OprM efflux pump.\textsuperscript{39} However, MexAB-OprM is the most commonly observed efflux pump system and also leads to fluoroquinolone, antipseudomonal penicillin, and antipseudomonal cephalosporin resistance. Without OprD loss, imipenem still retains susceptibility, as do aminoglycosides.\textsuperscript{20} Other efflux pumps noted to cause multidrug resistance include MexCD-OprJ, MexEF-OprN, and MexXY-OprM.\textsuperscript{19}

The other clinically significant mechanism of resistance to carbapenems within \textit{P. aeruginosa} is the production of a metallo-\(\beta\)-lactamase, a zinc enzyme of group 3, class B. Major metallo-\(\beta\)-lactamases include IMP, VIM, SPM, and GIM.\textsuperscript{15,60} Production of these enzymes can lead to resistance to all carbapenems plus the rest of the antipseudomonal \(\beta\)-lactams (e.g., ceftazidime, cefepime, piperacillin-tazobactam); however, aztreonam will retain activity. The \textit{P. aeruginosa} isolates that produce metallo-\(\beta\)-lactamases are more prevalent outside of the United States.\textsuperscript{61} For instance in Brazil and Italy, the production of a metallo-\(\beta\)-lactamase accounted for 43.9% and 39.1%, respectively, of the imipenem resistance to \textit{P. aeruginosa}.\textsuperscript{62} Fortunately, \textit{P. aeruginosa} isolates producing these enzymes are still rare in the United States. Sporadic cases or small outbreaks of isolates producing \textit{bla}\textsubscript{IMP} or \textit{bla}\textsubscript{VIM} have been reported in towns in Texas and New Mexico and in Chicago.\textsuperscript{63–66} The metallo-\(\beta\)-lactamases are inhibited by ethylenediaminetetraacetic acid (EDTA). Except for polymerase chain reaction analysis that identifies the specific \textit{bla}, only a few methods (including EDTA-containing Etest [AB Biodisk, Solna, Sweden] strips and the double-disk diffusion method) are available to clinical microbiology laboratories for nonspecific identification of the enzymes.\textsuperscript{67–69} Of note, the genes for these metallo-\(\beta\)-lactamases are carried on mobile gene cassettes inserted into class I integrons, which can be located chromosomally or on resident plasmids. As a result, these genes can be easily moved from one bacterium to another.

\textbf{Acinetobacter Species}

\textit{Acinetobacter} sp are nonfermenting gram-negative coccobacilli that are most commonly found as \textit{A. baumannii} in human infections.\textsuperscript{70} \textit{Acinetobacter baumannii} are usually commensal in healthy individuals and infrequently cause infections in the community setting; however, nosocomially acquired \textit{Acinetobacter sp} can be associated with pneumonia, skin infections, urinary tract infections, peritonitis, meningitis, and, often, bacteremia.\textsuperscript{70–74} Recently, this pathogen has raised many concerns because of its multidrug-resistant profile and the limited array of therapeutic options.

Unlike \textit{P. aeruginosa}, which is intrinsically resistant to many antibiotics, \textit{Acinetobacter sp} can be either exquisitely susceptible or highly resistant to antibiotics. Therefore, susceptibility rates are highly subjective to specific hospital or unit outbreaks. Recently, the U.S. military reported an outbreak of multidrug-resistant \textit{Acinetobacter calcoaceticus-baumannii} complex among injured military personnel deployed to Iraq and Afghanistan at two military medical centers.\textsuperscript{75,76} In one of these reports, among 142 isolates (84 wound infections) tested against 13 antibiotics, only three agents (polymyxin B, colistin, and minocycline) showed both high susceptibility (\(\geq 97\%\)) and high potency (MIC for 90% of tested strains [MIC\textsubscript{90}] \leq 4 \(\mu\)g/ml); meanwhile, greater than 30% of the isolates were nonsusceptible to imipenem (MIC\textsubscript{90} > 16 \(\mu\)g/ml).\textsuperscript{76} However, in the other report, 75 isolates were tested (40 bloodstream infections), and high rates of resistance were observed to nearly all agents tested except the carbapenems (20–25%).\textsuperscript{75}

In the United States, concern for multidrug-resistant \textit{A. baumannii} or \textit{A. calcoaceticus-baumannii} complex varies by region.\textsuperscript{34} In general, antibiotic susceptibilities were lower in the south-central (Kentucky and Alabama) and mid-Atlantic (New York and Pennsylvania) regions. For example, in various New York City hospitals, there were reports that a large percentage of \textit{A. baumannii} strains were not susceptible to many antipseudomonal agents (e.g., meropenem susceptibilities \leq 50\%).\textsuperscript{77,78} Table 3 provides the national susceptibility rates for \textit{Acinetobacter sp} against selected antibiotics tested during the 2005 MYSTIC study. Among the antibiotics tested, only imipenem and tobramycin had susceptibility rates greater than 90%.\textsuperscript{38} A separate surveillance study conducted in 2001 observed that 32.5% and 24.2% of \textit{A. baumannii} collected from intensive care and non-intensive care patients, respectively, exhibited multidrug resistance.\textsuperscript{32} The frequency
at which *Acinetobacter* sp is becoming multidrug resistant is especially disturbing when compared with the rates of multidrug resistance in *P. aeruginosa* isolates collected over the same time span; only 7% and 9.1%, respectively, of isolates from non–intensive care and intensive care patients were found to be multidrug resistant.

Like *P. aeruginosa*, *Acinetobacter* sp can become antibiotic resistant through numerous mechanisms, including β-lactamase production, outer membrane protein changes, production of aminoglycoside-modifying enzymes, mutations in topoisomerase, as well as efflux.\(^7^9\) Carbapenem resistance within *Acinetobacter* sp is often linked to reduced expression of several outer membrane proteins (all different than OprD in *P. aeruginosa*), along with the production of various β-lactamases.\(^8^0\) These include both serine and metallo-β-lactamases, the most significant of which are the OXA, IMP, and VIM-type enzymes. Again, these enzymes are most commonly found outside the United States. All *Acinetobacter* sp produce the chromosomally encoded AmpC cephalsporinase, which accounts for much of the resistance documented against cephalosporins.\(^8^1\) However, there is no evidence to suggest that this cephalsporinase is inducible.\(^8^2\) In addition to AmpC, OXA, IMP, and VIM, *Acinetobacter* sp can also produce TEM, SHV, and CTX-M-type ESBLs.

### Antibiotic Treatment Options

Table 4 displays antibiotic options for multidrug-resistant gram-negative bacilli by organism and resistance mechanism. The antibiotic choices listed are based on the authors’ own experiences, as well as a review of the available in vitro data, clinical studies, and case reports and consideration of potential for toxicity.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Clinical Resistance Mechanism</th>
<th>First-Line Options</th>
<th>Other Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>ESBL producing</td>
<td>Imipenem or meropenem</td>
<td>Ertapenem, tigecycline, aminoglycosides, fluoroquinolones (only for urinary tract infections)</td>
</tr>
<tr>
<td><em>Klebsiella sp</em></td>
<td>AmpC producing</td>
<td>Cefepime</td>
<td>Imipenem, meropenem</td>
</tr>
<tr>
<td></td>
<td>ESBL producing</td>
<td>Imipenem or meropenem</td>
<td>Ertapenem, tigecycline, aminoglycosides, fluoroquinolones (only for urinary tract infections)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>KPC producing</td>
<td>Tigecycline or polymyxins</td>
<td>Aminoglycosides, tetracyclines</td>
</tr>
<tr>
<td></td>
<td>Carbapenemase producing</td>
<td>Cefepime, meropenem, piperacillin-tazobactam, imipenem</td>
<td>Ceftazidime, aminoglycosides, fluoroquinolones (ciprofloxacin or levofloxacin)</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>Carbapenemase producing</td>
<td>Polymyxins</td>
<td>Aminoglycosides, aztreomycin (if a metallo-β-lactamase)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Imipenem or meropenem</td>
<td>Fluoroquinolones, doxycyclines, aminoglycosides, ampicillin-sulbactam</td>
</tr>
</tbody>
</table>

**ESBL** = extended-spectrum β-lactamase.

Carbapenems

In general, carbapenems are reserved at most hospitals for the sickest patients or for those who are infected with multidrug-resistant gram-negative bacilli. The carbapenem class includes imipenem-cilastatin, meropenem, ertapenem, and doripenem, the latter of which has recently been approved by the United States Food and Drug Administration (FDA). These antibiotics have broad-spectrum gram-positive and gram-negative activity, including activity against *P. aeruginosa* and *Acinetobacter* sp, with the exception of ertapenem, which lacks reasonable activity against nonfermenting gram-negative bacilli. They are also considered to be the antibiotics of choice for nonurine ESBL infections.\(^8^2\) Several studies have demonstrated improved efficacy or decreased mortality with imipenem or meropenem compared with other classes of antibiotics against ESBL-producing infections.\(^8^3\) This β-lactam class is particularly stable to hydrolysis by the ESBL enzymes. Although ertapenem is a carbapenem and has in vitro activity similar to meropenem against ESBL-producing *K. pneumoniae* and *E. coli*,\(^8^3\) some
In three case reports, complex collected in Spain, colistin Polymyxin resistance was still present. From a safety perspective, the clinical efficacy of these antibiotics against Acinetobacter and P. aeruginosa infections (primarily pneumonia) has also been observed in several small retrospective and prospective studies. Polymyxin resistance within P. aeruginosa and Acinetobacter sp is still quite rare, although with the continued required use of these antibiotics for these indications, it is not surprising that reports are emerging.

Of concern with use of these antibiotics is the limited information on pharmacokinetics, pharmacodynamics, standardization of dosing, and safety. Pharmacodynamic studies suggest that the polymyxins display concentration-dependent, rapid bactericidal activity. As such, tremendous confusion still exists on how to best dose these antibiotics, as well as the proper terminology to use when recommending dosages. From a safety perspective, polymyxins are known to cause nephrotoxicity and neurotoxicity; however, the exact frequency of these adverse events is also not well established. Historically, nephrotoxicity and neurotoxicity were reported at rates of 20% and 10%, respectively; however, newer studies suggest these rates may have been overstated because of inappropriate dosing, especially in patients with renal dysfunction.

Polymyxin B and Polymyxin E
Polymyxins are older agents that have extensive gram-negative coverage but have been flawed with risks of nephrotoxicity and neurotoxicity. Polymyxins consist of several different chemical forms but polymyxin B and E (colistin) are the two clinically used intravenous formulations. Of the two, colistin (colitimethate sodium) is primarily used because of fewer toxicities. According to the Clinical and Laboratory Standards Institute, colistin (and polymyxin B) susceptibility and resistance breakpoints are 2 µg/ml or less and 4 µg/ml or more, respectively. Colistin has been shown to be active against most of the troubling multidrug-resistant gram-negative bacilli (A. baumannii, P. aeruginosa, Klebsiella sp, E. coli). When susceptibilities were tested in 221 isolates of A. calcoaceticus-baumannii complex collected in Spain, colistin was found to have the highest rates among all the other antibiotics tested, including imipenem, meropenem, and sulbactam. Polymyxin B was also shown to have susceptibility rates 30% higher than those of any other antibiotic against 419 multidrug-resistant A. baumannii in New York City. The clinical efficacy of these antibiotics against Acinetobacter and P. aeruginosa infections (primarily pneumonia) have also been observed in several small retrospective and prospective studies.

Polymyxins consist of several different chemical producers. ESBL producers when compared with non-ESBL producers. We believe more clinical data are required before ertapenem can be uniformly recommended as a first-line option for ESBL-producing Enterobacteriaceae.

Meropenem and imipenem are routinely used as therapy for P. aeruginosa and A. baumannii infections. Between the two carbapenems, meropenem is noted to be more potent against P. aeruginosa and imipenem is more potent against Acinetobacter sp. In certain cases, as many as 31% of imipenem-resistant P. aeruginosa demonstrated susceptibility to meropenem. However, at most hospitals in the United States, susceptibility rates for these antibiotics are often comparable against this gram-negative organism (5–8% difference in susceptibility). Some in vitro studies have suggested lower MIC values with doripenem compared with meropenem or imipenem against P. aeruginosa, which may result in some strains being susceptible to doripenem when otherwise intermediate or resistant to the other agents. The clinical utility of doripenem against meropenem- or imipenem-resistant isolates, however, remains to be determined. Nonetheless, the carbapenems will usually be among the first therapeutic options for these multidrug-resistant gram-negative bacilli, when they still retain susceptibility.
Tigecycline

Tigecycline is a glycyclline derivative from minocycline that has in vitro activity against gram-negative organisms including MRSA, gram-negative organisms including ESBL-producing E. coli, Klebsiella sp, and Acinetobacter sp, and intracellular and anaerobic bacteria. It is indicated for complicated intraabdominal and skin and soft tissue infections. According to the FDA, tigecycline susceptibility and resistant breakpoints are 2 µg/ml or less and 8 µg/ml or greater, respectively, for gram-negative bacteria. Although tigecycline is a broad-spectrum agent, P. aeruginosa, Proteus sp, Providencia sp, Burkholderia sp, and Morganella sp are commonly resistant to the antibiotic. The predominant mechanism of resistance carried by some of these bacteria (i.e., P. aeruginosa and Proteus sp) is chromosomal-mediated efflux. Contrary to other antibiotics (i.e., aminoglycosides, quinolones, trimethoprim-sulfamethoxazole, and β-lactams), tigecycline appears not to be sensitive to cross-resistance. For instance, one study observed tigecycline to have a 4–256-fold greater activity compared with other antibiotics relinquished by ESBL-producing Enterobacteriaceae; most ESBL-producing isolates featured CTX-M. At 97.5%, the resulting susceptibilities of tigecycline were similar to the carbapenems tested and close to 30% greater than the other antibiotics that appeared to show co-resistance. Despite ESBL-producing isolates that expressed tetracycline resistance, tigecycline maintained its overall activity. Typically, with ESBL-producing isolates, tigecycline has shown remarkable activity similar to the agents of choice (i.e., carbapenems). When tigecycline was tested for in vitro activity against KPC, metallo-β-lactamase, and ESBL-producing Klebsiella sp and/or E. coli, it demonstrated 100% susceptibility. However, little data are available in the literature regarding the treatment of KPCs with tigecycline. A single case report of a patient with nosocomial pneumonia and empyema has been described. The patient was treated multiple times with prolonged courses of tigecycline. Subsequently, the patient failed therapy and died; recurrences of KPC in the empyema and a tigecycline MIC elevation from 0.75 to 2 µg/ml in the pleural fluid were found. Further data are required to support the use of this agent for serious infections caused by KPC-producing organisms.

In addition, although no susceptibility breakpoint is yet defined, in vitro studies have observed tigecycline activity against A. baumannii, and in some reports, activity better than the carbapenems. In the United States, tigecycline demonstrated the greatest potency of any agent tested (polymyxins were not tested in the study). However, with MIC₉₀ and susceptibilities rates (at a breakpoint of ≤ 2 µg/ml) for tigecycline having been 2 µg/ml or less and 95% or greater, respectively, the polymyxins still have greater potency (≤ 1 µg/ml) and susceptibility rates (97–99%) overall. Recent clinical data (case reports and case series) for the use of tigecycline in Acinetobacter infections have demonstrated mixed results. In one case report, successful treatment was observed with tigecycline, high-dose meropenem, and polymyxin in a patient with septic shock and pancreatitis, infected with multidrug-resistant A. baumannii. In a case series, clinical resolution was observed in 21 of 25 patients with ventilator-associated pneumonia and/or bacteremia caused by multidrug-resistant A. baumannii after the use of tigecycline as monotherapy or combination therapy. Contrary to these data, two other case reports demonstrated failure with the use of tigecycline in patients with various types of multidrug-resistant A. baumannii infections, with tigecycline MICs ranging from 4–24 µg/ml. In one of those case reports, the patient was empirically treated for 14 days with tigecycline (baseline MIC 1.5 µg/ml) for a urinary tract infection caused by multidrug-resistant A. baumannii. Twenty days after the end of therapy, the patient developed a paraspinal abscess and spinal osteomyelitis infected with A. baumannii; follow-up urine and sputum cultures grew A. baumannii, all with tigecycline MICs of 24 µg/ml.

Recently, an in vitro report in Israel showed that nosocomial-acquired A. baumannii isolates resistant to aminoglycosides, cephalosporins, and fluoroquinolones and mildly susceptible to imipenem (73%) may be resistant to tigecycline. The results found tigecycline to have only 22% susceptibility with an MIC₉₀ and MIC₅₀ of 16 and 32 µg/ml, respectively. In addition, two cases of tigecycline-resistant A. baumannii bacteremia were reported in patients being treated with the drug for other bacterial infections. These reports bring to light that although tigecycline may have in vitro activity against many Acinetobacter sp, clinical success may more importantly depend on the source of infection; the drug has an enormous volume of
distribution, and thus blood concentrations may be below the MIC against many of these multidrug-resistant \textit{Acinetobacter} sp.

\textbf{Sulbactam}

Sulbactam is a \beta-lactamase inhibitor that is formulated in combination with the \beta-lactam, ampicillin; however, in countries other than the United States, sulbactam is also found in combination with a cephalosporin (cefeoperazone). In vitro studies have shown sulbactam to have favorable bactericidal activity against \textit{A. baumannii}.\cite{126} Although susceptibility and potency have not been as high as that of colistin in \textit{A. calcoaceticus-baumannii} complex, sulbactam has been shown to have greater potency and/or susceptibility than that of meropenem and imipenem.\cite{93} When used in combination with other \beta-lactams such as cefepime and meropenem, it has shown moderate in vitro synergistic activity based on the checkerboard method to \textit{A. baumannii}.\cite{127, 128} Of note, in vitro resistance to ampicillin-sulbactam may not correlate with clinical outcomes since the concentrations of sulbactam tested are usually below that which can be achieved with clinical doses.\cite{129}

Clinical success of ampicillin-sulbactam has been documented for the treatment of \textit{A. baumannii}, as well as multidrug-resistant strains. In one study, 39 of 42 patients with multidrug-resistant \textit{A. baumannii} improved or were cured after treatment with ampicillin-sulbactam or sulbactam alone.\cite{130} In cases in which patients had ventilator-associated pneumonia with \textit{A. baumannii} infection, treatment with ampicillin-sulbactam was effective.\cite{131} In other cases of multidrug-resistant \textit{A. baumannii}, high-dose regimens of sulbactam 8 g/day were used for the treatment of ventilator-associated pneumonia.\cite{132, 133} In one prospective study, 7–10 days of monotherapy with high-dose ampicillin 18 g–sulbactam 9 g versus ampicillin 24 g–sulbactam 12 g was compared in patients isolated with ventilator-associated pneumonia with multidrug-resistant \textit{A. baumannii} infection.\cite{133} Of the 27 patients in the study, success was achieved clinically (64.3\% and 69.2\%, \(p=0.785\)) and bacteriologically (85.7\% and 69.2\%, \(p=0.3\)) in both groups. The 14- and 30-day mortality rates were 25.9\% and 48.1\%, respectively, whereas the frequency of adverse reactions was 14.8\%, with one patient having transient renal deterioration. Of interest, all 27 \textit{Acinetobacter} sp were reported as resistant. As a result of these data, ampicillin-sulbactam may be a safe and effective agent against multidrug-resistant \textit{Acinetobacter} sp, but when the drug is administered as monotherapy, data are still limited and the consequence of in vitro resistance is still not well elucidated.

\textbf{Combination Therapy}

The principle of using two agents of different classes to improve coverage, provide a synergistic or additive effect, or prevent the emergence of resistance is commonly given as support for combination therapy. This principle is notably applied with the use of aminoglycosides or fluoroquinolones with a \beta-lactam (i.e., piperacillin-tazobactam, cefepime, ceftazidime, imipenem, or meropenem). These concepts have been reviewed in detail elsewhere.\cite{134} Nevertheless, it is likely that the multidrug-resistant gram-negative bacilli highlighted herein may often require combinations of drugs in an attempt to optimize empiric therapy. In many of the case reports presented throughout this review, at least two antibiotics were used to attain a clinical response, even if one was reported as non-susceptible. Clearly, decisions surrounding which combination of antibiotics should be used will depend on several factors: in vitro susceptibility, severity of infection, drug allergies, potential for adverse events, or even comfort level of the clinician.

Most data supporting synergy against multidrug-resistant \textit{P. aeruginosa}, \textit{Acinetobacter} sp, and other nonfermenting gram-negative organisms (e.g., \textit{Burkholderia cepacia} complex) is based in the cystic fibrosis literature.\cite{135, 136} Unfortunately, these data have never been shown to be predictive of clinical or microbiologic response. At times, nontraditional combinations of antibiotics might be necessary to treat some of these multidrug-resistant strains. For example, combinations of colistin with vancomycin or rifampin have been shown to produce synergistic lowering of the MICs against some KPC-producing \textit{Klebsiella} strains and have been used successfully in some cases. The membrane-disrupting effects of colistin theoretically allow antibiotics like vancomycin to demonstrate activity against gram-negative organisms.

\textbf{Pharmacodynamic Considerations}

Other answers for the treatment of multidrug-resistant gram-negative bacilli might be found
within the antibiotics that we still have available, particularly if the exposure-response relationship (i.e., pharmacodynamics) is considered. This topic is reviewed in detail elsewhere.\textsuperscript{137} For the β-lactam antibiotics, optimization of pharmacodynamics can be achieved with a combination of dose increases as well as modifications to the infusion duration, such that the requisite free drug time above the MIC (\textit{fT>MIC}) can still be achieved, even if the antibiotic MIC is elevated beyond susceptible against the pathogen.\textsuperscript{138}

The successful utilization of prolonged infusion β-lactam regimens, particularly meropenem and piperacillin-tazobactam, have been observed against multidrug-resistant gram-negative infections causing pneumonia, meningitis, and other serious infections.\textsuperscript{139–141} In one case report, a dose of meropenem 2000 mg every 8 hours administered over 3 hours achieved 40% \textit{fT>MIC} in serum against an isolate of \textit{B. cepacia} with a meropenem MIC of 16 µg/ml; the patient responded positively to the regimen.\textsuperscript{139} In a retrospective study, application of a prolonged infusion of piperacillin-tazobactam for \textit{P. aeruginosa} infections resulted in decreased mortality among patients with higher Acute Physiology and Chronic Health Evaluation II scores.\textsuperscript{141} The piperacillin-tazobactam dosage used by their hospital was 3.375 g every 8 hours as a 4-hour infusion, which was expected, based on Monte Carlo simulation models, to improve the likelihood of attaining bactericidal exposure for organisms with MICs of 16 and 32 µg/ml, respectively, which are still considered susceptible yet poorly attainable with standard piperacillin-tazobactam dosing.

These dosing strategies were tested in a simulation model against multidrug-resistant \textit{P. aeruginosa} from Hungary; application of larger dosages and prolonged infusions significantly increased the likelihood of pharmacodynamic target attainment for ceftazidime, meropenem, and piperacillin-tazobactam regimens.\textsuperscript{142} Further clinical data are required to determine a better understanding of how these pharmacodynamically designed dosage regimens will be expected to perform in light of in vitro resistance.

**Conclusion**

The steady trend of resistance has left many health care professionals with few options in the treatment of gram-negative bacilli. Although multidrug-resistant \textit{P. aeruginosa} and \textit{Acinetobacter} sp are common in hospitals across the United States, resistance mechanisms such as metallo-β-lactamases that make these bacteria essentially panresistant are still fortunately rare. Meanwhile, the rates of ESBL production in \textit{E. coli} and \textit{Klebsiella} sp continue to rise, and with the need for carbapenem therapy to treat these infections, the potential for emerging resistance to this class is worrisome. Currently, carba-penemase production in these Enterobacteriaceae (i.e., KPC) are regional and infrequent. In light of emerging gram-negative resistance combined with an empty pharmaceutical pipeline in the foreseeable future, we must make appropriate use of both newer (e.g., tigecycline, doripenem) and older (e.g., polymyxins, ampicillin-sulbactam) antibiotics so that therapeutic options are still available for years to come. It is plausible that the treatment of infections caused by these bacteria will soon require combinations of antibiotics and individual dosages based on pharmacodynamics to achieve requisite exposures at higher MICs. Finally, and although not discussed in this review, antibiotic stewardship and adequate infection control practices should be reinforced in order to control the further spread of these nearly untreatable gram-negative bacteria.

**References**


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