The Emergence of Carbapenemase-Producing Enterobacteriaceae

Enterobacteriaceae encompass a large group of commensal bacteria in the human bowel, including Klebsiella pneumoniae and Escherichia coli, which are common etiologies of both community- and hospital-acquired infections. Nosocomial Klebsiella isolates have exhibited increasing levels of antibiotic resistance over the past 20 years.

This process has been facilitated by the fact that genes that encoded antibiotic-resistance determinants were often located on plasmids that were transferred with high frequency between K. pneumoniae strains and other genera of Enterobacteriaceae. This process is best epitomized by the worldwide emergence of extended-spectrum β-lactamases (ESBLs) and their rapid dissemination among Enterobacteriaceae—especially K. pneumoniae strains.

Although ESBLs are often referred to as a single entity, they actually are a complex group of enzymes capable of hydrolyzing a wide variety of β-lactam substrates. Each ESBL has some degree of unique regional distribution across the world. For instance, TEM- and SHV-type ESBLs are common in the United States, whereas CTX-M-type ESBLs are more common throughout Asia, Europe, Canada, and South America. The β-lactamase genes that encode for each ESBL (eg, blaTEM, blaSHV) often are linked on plasmids with genes that produce aminoglycoside- or quinolone-resistance factors. Plasmid transfer (as depicted in Figure 1) can result in extensive antibiotic resistance,1,2 and this phenomenon has led to an increased reliance on carbapenems in clinical practice.3

By 1995, ESBLs commonly were encountered among Klebsiella isolates, but carbapenem resistance was relatively uncommon and primarily was the result of the combined activity of a weak AmpC β-lactamase and porin mutations; less frequently, it could be attributed to species-specific, chromosomally-encoded β-lactamases that could hydrolyze carbapenems.1 In 1995, there was an ominous report from Japan about a plasmid-borne gene recovered from a Serratia isolate that encoded for a metallo–β-lactamase gene called IMP-1, which was capable of hydrolyzing carbapenems.4 In 2001, a similar report from North Carolina documented the identification of a plasmid-borne gene, KPC-1, encoding for a non-metallo–β-lactamase capable of hydrolyzing carbapenems.
Carbapenemases that was recovered from a *K. pneumoniae* isolate. Since then, the number of newly recognized plasmid-borne carbapenemases has proliferated and they have rapidly disseminated to become a global problem. Although referred to as carbapenemases, they possess the ability to hydrolyze a broad variety of β-lactams, including carbapenems, cephalosporins, penicillins, and sometimes, aztreonam. When linked to other resistance factors on the same plasmid, they can present a significant therapeutic challenge to clinicians.

**Classification and Epidemiology of Carbapenemases**

Carbapenemases identified in *Enterobacteriaceae* belong to 3 classes of β-lactamases based on molecular characteristics: Ambler class A, B, and D. A brief discussion of each class follows, with a focus on the plasmid-borne carbapenemase genes.

Class A carbapenemases include *K. pneumoniae* carbapenemases (KPC), IMI-2, and GES (Guiana extended-spectrum β-lactamases)—all of which effectively hydrolyze carbapenems and all other β-lactams, have serine active sites, and are partially inhibited by clavulanic acid. Plasmid-encoded KPCs have emerged as the most clinically dominant member of the class. First described in 2001 in North Carolina, KPC subsequently was detected in 2004 during multiple hospital outbreaks in New York City; by 2007, it had emerged in multiple hospitals on the East Coast. KPC became endemic in New York City and areas of New Jersey and now has been reported in 38 states across the country. Similarly, KPC rapidly disseminated to France, Israel, Greece, Colombia, and China, and reported outbreaks of KPC have occurred in many European countries, South America, and India. KPCs are encoded by the *bla*KPC gene, which is located within a Tn3-type transposon, Tn4401, and is capable of inserting into diverse plasmids of gram-negative bacteria. There are now 11 known variants of the KPC gene, *bla*KPC-1 through *bla*KPC-12, although the global dissemination of KPC is largely associated with KPC-2 and KPC-3—KPC-1 and KPC-2 subsequently were shown to be genetically identical. KPCs have largely emerged among nosocomial *K. pneumoniae* isolates, but they have been reported with some frequency among nosocomial isolates of *E. coli*, *Enterobacter* species, *S. marcescens*, *Citrobacter freundii*, and rarely, *Pseudomonas* and *Acinetobacter*; KPC isolation has been associated with both acute- and long-term care facilities; community isolates rarely are reported. KPCs have demonstrated a close association with individual *K. pneumoniae* clones. Sequence type (ST)-258 has been extensively identified across the world and appears to be associated with the global dissemination of KPCs.

Class B carbapenemases include Verona integron-encoded metallo-β-lactamases (VIM), imipenem-hydrolyzing β-lactamases (IMP), and New Delhi metallo-β-lactamase (NDM-1). These enzymes hydrolyze all β-lactams except aztreonam, have zinc-active sites, and are inhibited by ethylenediaminetetraacetic acid (EDTA), but not by clavulanic acid. IMP was first reported in 1991 in a *Pseudomonas* isolate from Japan. VIM was first reported in 1997 in a *Pseudomonas* isolate from Italy. Subsequently, both enzymes gradually moved into a number of *Enterobacteriaceae* genera. Both VIM and IMP now are endemic in Greece, Italy, Spain, Taiwan, and Japan, although outbreaks and increasing reports of the isolates have come from Europe.

Cases of both IMP and VIM have been noted in many other countries, but they are only occasionally reported in the United States. VIM and IMP carbapenemases now are largely reported from nosocomial *Klebsiella* isolates that exhibit multiple antibiotic resistance patterns—both are associated with integron elements that facilitate their insertion into plasmids. There are 21 known variants of IMP and 24 variants of VIM have been described. VIM has been clustered into 3 lineages: VIM-1, VIM-2, and VIM-7.

The most prominent and problematic member of this class is NDM-1, which is encoded by the carbapenemase gene *bla*NDM-1 (Figure 2). This enzyme was first described in 2008 in a *Klebsiella* isolate obtained from a patient in Sweden who previously had been hospitalized in New Delhi, India. By 2010, *bla*NDM-1-containing *Enterobacteriaceae* had been reported in every continent except Central and South America, and a direct link to the Indian
Subcontinent (i.e., India, Pakistan, and Bangladesh) was established for most of these cases. Subsequent investigations revealed that the bla<sub>NDM-1</sub> gene was present on the plasmids of numerous clinical isolates from both nosocomial- and community-acquired <i>K. pneumoniae</i> and <i>E. coli</i> infections that were obtained from a wide geographic area of the Indian subcontinent. NDM-1 carriage also was documented among numerous other species of <i>Enterobacteriaceae</i>. Both community and nosocomial isolates exhibited multiple antibiotic resistance patterns, and NDM-1 carriage was identified in multiple bacterial clones. After investigators evaluated seepage water and public tap water samples within a 12-km radius of New Delhi, they identified NDM-1–positive strains of <i>K. pneumoniae</i>, <i>E. coli</i>, and <i>C. freundii</i>, as well as <i>Shigella boydii</i>, <i>Vibrio cholera</i>, and a variety of nonfermentative gram-negative bacteria. It is now believed that the Balkan countries, the Middle East, and possibly, the United Kingdom, also are endemic reservoirs for NDM-1.

Class D carbapenemases include oxacillin-hydrolyzing metallo–β-lactamases (OXA), of which OXA-48 is the most common. These enzymes are somewhat unique because they exhibit only a weak ability to hydrolyze carbapenems, broad-spectrum cephalosporins, and aztreonam. High-level carbapenem resistance occurs only when OXA enzymes are coexpressed with ESBLs and porin resistance factors. Class D carbapenemases have active serine sites and are not inhibited by clavulanic acid or EDTA. OXA–β-lactamase genes that encode for <i>blaoxa-48</i> most often are recovered from nosocomial isolates of <i>K. pneumoniae</i> and <i>E. coli</i>.

OXA-48 was first identified in a <i>K. pneumoniae</i> isolate from Turkey in 2003; since then, it has become endemic there and also has disseminated across Europe, the Southeast Mediterranean region, and Africa. A second variant, OXA-181, has been reported in India. OXA genes have not been reported in the United States, but the epidemiology of OXA carbapenemases continues to evolve.

Carbapenemases are a complex group of β-lactamases that display significant genetic diversity, but they all share several troubling characteristics. First, <i>Enterobacteriaceae</i> strains that carry mobile carbapenemases often express multiple antibiotic-resistance patterns, thus creating a therapeutic challenge. Second, they all exhibit high variability regarding which carbapenems they hydrolyze, a trait that makes it difficult to identify them with phenotypic testing. Similarly, the extensive genetic diversity among the types of carbapenemases—and the diversity within each type—can present a significant challenge to identify them with available molecular techniques.

**Laboratory Identification of Carbapenemases**

Current methods for identifying carbapenemase-producing bacteria are based on disk diffusion or, increasingly, automated microdilution susceptibility testing. Although the Clinical and Laboratory Standards Institute reduced the break points for carbapenems in 2010 to improve carbapenemase detection in the United States, these approaches still will not reliably detect all instances of whichever carbapenemase may be present. Specific tests have been used to supplement disk diffusion and automated microdilution when high-level resistance is not noted. The modified Hodge test has been used to screen for carbapenemase production in this setting (Figure 3), especially for KPC; however, it lacks specificity (false-positives from AmpC producers) and sensitivity. Additionally, it does not reliably identify NDM-1. A variety of other tests using EDTA and boronic acid inhibition have been investigated, but they have not reliably demonstrated the requisite sensitivity and specificity. Similarly, selective disclosing media have been developed to screen isolates for carbapenemase production in...
both clinical and infection-control settings. Several methods have been commercialized, including CHROMagar KPC, Brilliance CRE (Oxoid, Ltd.), and HardyCHROM carbapenemase (Hardy Diagnostics). These approaches also have documented issues with sensitivity and specificity.\textsuperscript{21,22} Additionally, these secondary tests are labor-intensive, expensive, not well suited for high-throughput testing, and add at least an additional 18 hours to specimen turnaround time.\textsuperscript{21,22} Currently, there is no phenotypic-based diagnostic test that has demonstrated the required sensitivity, specificity, and ease of adaptability to the clinical laboratory that can rapidly identify all known types of carbapenemases.\textsuperscript{20,21}

In contrast, molecular diagnostic approaches rapidly are becoming the gold standard for the detection of carbapenemases. Initial efforts were directed at the creation of assays that could be used in support of active surveillance programs that drive infection control interventions. They primarily consisted of a variety of “homegrown” real-time multiplex polymerase chain reaction (PCR) assays that were optimized for rectal swab KPC detection.\textsuperscript{23,24} The use of an experimental prototype real-time multiplex PCR assay (Becton, Dickinson and Company) with primers for all known variants of KPCs was recently described.\textsuperscript{25} A single commercial product is available, Hy-KPC PCR (Hy Laboratories, Ltd). These assays were shown to be highly sensitive and specific with 2-hour turnaround times and, when combined with intensive bundled infection-control interventions, they have proven to successfully limit the prevalence of KPCs on an institutional, regional, and national basis.\textsuperscript{26-30} This approach is very promising for areas of the world where KPCs currently are the predominant carbapenemase, such as in the United States. Similarly, both real-time PCR assays and a loop-mediated isothermal amplification assay have targeted NDM-1 detection.\textsuperscript{31} Only a handful of these assays have been commercialized and none of them currently are FDA approved.

Recognition of the continuing trend toward the globalization of carbapenemases and the need to address regions where several types already are in circulation (eg, India, Greece, and Europe) have driven the development of a variety of molecular diagnostic assays that can detect all types of carbapenemases. These include DNA microarray and multiplex real-time PCR assays that can detect the genetic elements of KPC, NDM-1, IMP, VIM, and OXA-48.\textsuperscript{32-33} The Check-MDR CT102 DNA microarray (Check-Points BV) has been commercialized, but currently is not FDA approved. Despite the great promise that current molecular assays have demonstrated regarding sensitivity, specificity, detection of multiple carbapenemase types, and rapid turnaround time, it remains to be seen if they can be adapted to meet the demands required for routine clinical diagnostic use. Additional issues include the need for automation with minimal sample processing, high throughput, cost, and a flexibility that will accommodate the ever-increasing genetic diversity of carbapenemases.\textsuperscript{2} Both the treatment and containment of carbapenemase-producing Enterobacteriaceae rapidly have emerged as a significant threat to medical practice. They are becoming the gold standard for the detection of carbapenemase-producing Enterobacteriaceae, which now are routinely exhibiting resistance to multiple antibiotics. Although they often are used in combination with other antibiotics, this approach seems to offer little advantage over the use of high-dose polymyxin alone.\textsuperscript{2,36}

However, combination therapy with tigecycline may be important in preventing the development of polymyxin resistance, which has been reported to arise during therapy (and has increasingly been reported in general).\textsuperscript{2,36} Tigecycline is a glycyclline antibiotic that frequently is reported to have activity against carbapenemase-producing bacteria. However, the drug does not reliably achieve adequate levels in urine or blood and, hence, is not indicated for the treatment of urinary tract infections, serious bacteremias, pneumonia, or central nervous system infections.\textsuperscript{36} A recent FDA alert recommended the use of alternative agents to tigecycline in the face of serious infections following evidence of increased mortality associated with tigecycline treatment in this setting.\textsuperscript{36} Tigecycline increasingly is being relegated to the role of a last-resort treatment option.

Fosfomycin susceptibility is observed in more than 50% of carbapenemase-producing Enterobacteriaceae.\textsuperscript{36} The drug acts by inhibiting early stages of cell wall synthesis and is available in granular form for use as an orally administered liquid. Fosfomycin is useful for the treatment of urinary tract infections, and further investigations are required to determine if there is any other role for this antibiotic.\textsuperscript{36} Fosfomycin resistance frequently emerges during treatment and it has been suggested that it should always be used in combination with another drug.\textsuperscript{36}

Given the limited number of drugs in the gram-negative development pipeline, it is important to focus on interventions that reduce the prevalence of infections caused by carbapenemase-producing Enterobacteriaceae. Additional efforts will need to focus on the prudent use of what limited antibiotics currently are available. Both strategies may prove useful in preventing the development of resistance to current treatment options.

**Conclusion**

The carbapenemase-producing Enterobacteriaceae rapidly have emerged as a significant threat to medical practice. They are becoming the gold standard for the detection of carbapenemase-producing Enterobacteriaceae, which now are routinely exhibiting resistance to multiple antibiotics. Although they often are used in combination with other antibiotics, this approach seems to offer little advantage over the use of high-dose polymyxin alone.\textsuperscript{2,36} However, combination therapy with tigecycline may be important in preventing the development of polymyxin resistance, which has been reported to arise during therapy (and has increasingly been reported in general).\textsuperscript{2,36} Tigecycline is a glycyclline antibiotic that frequently is reported to have activity against carbapenemase-producing bacteria. However, the drug does not reliably achieve adequate levels in urine or blood and, hence, is not indicated for the treatment of urinary tract infections, serious bacteremias, pneumonia, or central nervous system infections.\textsuperscript{36} A recent FDA alert recommended the use of alternative agents to tigecycline in the face of serious infections following evidence of increased mortality associated with tigecycline treatment in this setting.\textsuperscript{36} Tigecycline increasingly is being relegated to the role of a last-resort treatment option.

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global health. There is an urgent need to develop effective diagnostic testing strategies to guide both timely clinical treatment and to maximize infection control interventions. Failure to address these issues is likely to be associated with an intensification of the problem. Current treatment options are limited and without effective control of the prevalence of these organisms and the prudent use of the limited antibiotics available, widespread resistance could develop.

References


Dr. Currie reported no relevant conflicts of interest.