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Carbapenemases

A Brief Review for Pediatric Infectious Disease Specialists

Gary D. Overturf, MD

Key Words: beta-lactamase or beta-lactamases, carbapenemase or carbapenemases, carbapenems, imipenem, meropenem, doripenem, antibiotic resistance

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Carbapenems are increasingly utilized against a variety of infections because of the emergence of bacteria producing extended spectrum beta-lactamases (ESBL) in the *Enterobacteriaceae*, particularly *Escherichia coli*, *Klebsiella pneumoniae*, and other enteric bacteria^{1,2} Carbapenems (imipenem, meropenem, ertapenem, and doripenem) are often the drugs of last resort for ESBL producing organisms which are increasingly also resistant to quinolones, aminoglycosides, trimethoprim-sulfamethoxazole and other antibiotics, thereby meeting the definition of multiply drug resistant organisms.³ In addition, the carbapenems are often relied upon for uniquely resistant isolates of *Pseudomonas aeruginosa* and *Acinetobacter* spp. However, the emergence and proliferation of bacteria producing carbapenemases are increasingly being seen in clinical prac-

tice, jeopardizing the effective use of carbapenems generating a whole new class of Gram negative “superbugs.”

Resistance to carbapenems may not always due to the production of carbapenemases⁴ Some resistance among *Enterobacteriaceae* are caused by the expression of AmpC type enzymes when combined with a limitation to cellular penetration via a porin loss, then carbapenem resistance can occur. In addition, other “conventional” beta-lactamases such as the SHV class of ESBLs with porin loss can also produce a phenotype of carbapenem resistance.⁵ However, this discussion will focus on the emerging issue of carbapenemases in clinical isolates and the hazards they pose in laboratory detection and effective clinical treatment of infections.

CARBAPENEMASES

Table 1 outlines the common carbapenemases produced by pathogenic bacteria. These enzymes fall into 3 of the Ambler classes of beta-lactamases, A, B, and D classes and include the *Klebsiella pneumoniae* carbapenemases (KPC), 4 serine carbapenemases (SME, NMC-A, IML, and rare GES) and several metallo-beta-lactamases (IMP, VIM).^{6,7} A last group of enzymes, OXA, are only weakly active against carbapenems and are largely confined to *Pseudomonas* and *Acinetobacter* species, and only rarely in *Enterobacteriaceae*.⁸ It is unknown whether OXA carbapenemases, which are confined

to bacterial chromosomes and not present on mobile elements, will emerge as significant causes of resistance in bacteria other than *Acinetobacter*.

KPC Carbapenemases

These agents are the most commonly occurring class A carbapenemases and yet have been found only recently.⁹ Although KPC 1–8 have been described, types 1 and 2 have been subsequently been found to be identical; the rest are variants of the *bla*_{KPC} genes on conjugative plasmids that often carry other resistance markers such as fluoroquinolone and aminoglycoside resistance. Interspecies transfers of these enzymes have been suggested in studies in some health care facilities. KPC enzymes when present are generally broadly active against all beta-lactams despite the fact that they may test susceptible to some carbapenems (particularly imipenem and meropenem) as well as to cefepime and cephamycins, particularly when using agar dilution methods such as disk testing and Etest.⁷ Some automated systems have been associated with this difficulty as well. However, ertapenem resistance generally has been found to be the single most sensitive indicator of carbapenem resistance with KPCs, but when dilution tests are performed, the minimum inhibitory concentration (MIC) of imipenem and meropenem will be found to be elevated, to at least the “intermediate” range of MIC.

KPC enzymes have been most often in *K. pneumoniae*, but like ESBLs these

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TABLE 1. Carbapenemases by Classification, Activity and Organisms

Enzyme Type	Classification by Ambler Class	Activity Spectrum	Organism(s)
KPC	A	All β -lactams	Enterobacteriaceae <i>Ps. aeruginosa</i>
SME	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	<i>S. marcescens</i>
NMC-A, IMI	A	Same as for SME	<i>Enterobacter</i> spp.
GES	A	Imipenem and 3rd/4th cephalosporins	<i>Ps. Aeruginosa</i> and Enterobacteriaceae
IMP, VIM	B (metallo- β -lactamases)	All β -lactams; can test susceptible to aztreonam	<i>Pseudomonas</i> spp. <i>Acinetobacter</i> spp. Enterobacteriaceae
OXA	D	Weakly active against carbapenems	<i>A. baumannii</i> , <i>P. Aeruginosa</i> , and rare Enterobacteriaceae

enzymes are no longer confined to this organism, and KPCs have been found in *Klebsiella oxytoca*, *Salmonella enterica*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter Cloacae*, and *Serratia marcescens*.⁷ In addition, they have been found in rare isolates of *Ps. aeruginosa* in Puerto Rico and Colombia. The first KPC isolates (*K. pneumoniae*) occurred in the United States in North Carolina and are now concentrated in New York, New Jersey, Maryland, Pennsylvania, but now rarely in Florida, Colorado, New Mexico, and California, as well as Missouri, Arkansas, Virginia, and Alabama.^{7,10} However, KPCs are now widely distributed worldwide with reports in Israel, China, Greece, South America and India.^{6,7}

Serine Carbapenemases

Class A serine carbapenemases are chromosomal enzymes including SME, IMI and NMC-A and plasmid borne enzymes, the GES beta-lactamases.^{6,11} Imipenem and cefoxitin induce chromosomal carbapenemases; conferring a unique susceptibility profile with resistance to carbapenems, penicillins, and aztreonam but susceptibility to

extended spectrum cephalosporins. The activity of these enzymes is susceptible to inhibition by clavulanate, but not sulbactam. The presence of these genetic elements on chromosomes and not on mobile genetic elements, is cited as the reason that intraspecies spread has been rare. These SME group are confined in *S. marcescens* and the GES enzymes are also rare, but are found as cassettes within integrons on plasmids mostly in *Ps. aeruginosa*.

Class B Metallo- β -Lactamases

Class B Metallo- β -lactamases (MBL) carbapenemases are of the Ambler class B and have a wide spectrum of activity against carbapenems, penicillins and extended spectrum cephalosporins but not aztreonam.^{6,7} These enzymes require zinc as a cofactor and they are inhibited by EDTA, a chelator of divalent cations. These enzymes occur in multiple genera of Gram negative bacteria including Enterobacteriaceae as well as non-fermenters. The enzymes are found world wide and like KPCs have spread rapidly, presenting a serious threat because of their prolific dissemination.¹² The VIM and IMP type of MBLs are the most common.

The VIM MBL consist of a family of 14 enzymes, but VIM-2 predominates in most outbreaks.

LABORATORY DETECTION OF CARBAPENEMASES

Detection of carbapenemase activity in Enterobacteriaceae is a challenge particularly for the most frequent enzymes of the MBL and KPC type (Table 2). These enzymes do not always produce resistant breakpoints for carbapenems, using standardized susceptibility testing methods. Effective treatment and infection control depend upon the rapid and efficient identification of these isolates. Unfortunately, carbapenem susceptibility by reference MIC methods, such as the broth microdilution and agar dilution, are more sensitive than disk diffusion, Etest, and many automated systems.⁷ However, although Enterobacteriaceae with KPC generally have higher MICs they may not test into the defined resistant range. MICs of ≥ 1.0 to $2.0 \mu\text{g/mL}$ against ertapenem, meropenem, or imipenem has been found to be an effective screen of the likely presence of

TABLE 2. Summary of Available Methods to Detect Carbapenemases in Clinical Isolates of Bacteria

Name of Test	Method	Result Indicating the Presence of a Carbapenemase	Use of Test
Screening with Susceptibility or Resistance to Carbapenems	Determination of MIC by E-Test or Automated System	Ertapenem MIC $\geq 1.0-2.0 \mu\text{g/mL}$; or Imipenem MIC $\geq 2.0-4.0 \mu\text{g/mL}$	Screen for the presence of KPC in Enterobacteriaceae (not definitive for any type of carbapenemase)
Tris - EDTA Enhanced Susceptibility	Tri-EDTA enhanced disk or E-Test MIC determination	Presence of Antibiotic/EDTA MIC ration of ≥ 8.0 vs. MIC without EDTA	Primarily used to confirm an MBL carbapenemase in an isolate resistant to carbapenems
Hodge Test	Disk diffusion in the presence of <i>E. coli</i> carbapenemase producing bacteria	Inhibition of zone of activity by test organism with standard disk(s) of carbapenem(s)	General screen for the presence of a carbapenemase, but cannot differentiate between MBL and KPC.
PCR for Genetic Elements for Resistance to Carbapenems	Example: PCR for <i>bla</i> _{KPC} (eg most common carbapenemase gene in U.S.)	Positive PCR for gene	Available only in research or select reference laboratories

KPCs, whereas MBLs produce MICs ≥ 2.0 $\mu\text{g/mL}$ against imipenem or meropenem. Therefore recommendations for testing have suggested that most MBL producers will have MIC for imipenem and meropenem greater than 2.0 $\mu\text{g/mL}$ and have suggested using this as a cutoff or cutoff ranging from 1 to 4 $\mu\text{g/mL}$ as a “screening” dilution for possible carbapenemase production.¹³ As mentioned previously still others suggest ertapenem resistance as the most sensitive screen with MICs of >1 to 2 $\mu\text{g/mL}$ as the most accurate way to detect KPC and MBL carbapenemases.

Once a screen criteria, such as a resistant MIC cutoff for ertapenem or imipenem has been selected, there are a number of phenotypic tests which have been developed to detect carbapenemases in Gram negative bacteria. The Modified Hodge Test is a relatively easily performed test on a single agar plate to detect both KPC and MBL enzymes, but it cannot differentiate between them.¹⁴ A standardized inoculum of a lawn of a reference *E. coli* is utilized against carbapenem disks on the isolates to be tested. Multiple isolates can be tested on a single agar and multiple antibiotics and it relatively easy to read, but is somewhat subjective. Several versions of an EDTA disk test⁷ have been used for detections for MBL carbapenemases including one which utilizes a double sided Etest with imipenem vs. imipenem with EDTA,¹⁵ a ratio of ≥ 8 between the MIC of the non-EDTA en-

hanced versus the EDTA enhanced imipenem MIC indicates the presence of a MBL beta-lactamase.

SUMMARY

Carbapenem resistance constitutes a serious threat to the antibiotics available to deal with increasing resistance in Gram negative pathogens infecting neonates, infants, and compromised children with nosocomial infection caused by carbapenemase and ESBL producing bacteria. The dissemination in hospitals and the location of these enzymes on highly mobile genetic elements has contributed to their rapid spread and the frequent cotransfer of multiple other antibiotic resistance factors. The ability to limit the spread of these pathogens will require effective laboratory screening methods to rapidly identify patients infected with these organisms. Although current criteria to screen for these enzymes and methods for confirmation are useful, laboratories will need new tools, perhaps molecular techniques, to make the process rapid and accurate.

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