

# The Problem of Carbapenemase-Producing-Carbapenem-Resistant-*Enterobacteriaceae* Detection

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**The emergence and spread of carbapenemase-producing carbapenem-resistant *Enterobacteriaceae* (CP-CRE) are a significant clinical and public health concern. Reliable detection of CP-CRE is the first step in combating this problem. There are both phenotypic and molecular methods available for CP-CRE detection. There is no single detection method that is ideal for all situations.**

Gram-negative bacteria, specifically *Enterobacteriaceae*, are common causes of both community-acquired and hospital-acquired infections, including urinary tract, bloodstream, and lower respiratory tract infections. These bacteria can acquire genes encoding multiple antibiotic resistance mechanisms, including extended-spectrum  $\beta$ -lactamases (ESBLs), AmpCs, and carbapenemases (1).  $\beta$ -Lactam drugs are often the primary therapeutic option for serious infections, and carbapenems in particular are often considered agents of last resort. Thus, the emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE) are a significant clinical and public health concern. CRE are often resistant to all  $\beta$ -lactam drugs and frequently carry mechanisms conferring resistance to other antimicrobial classes, further limiting treatment options. Although CRE infections are relatively infrequent, they have become more common in the United States since their emergence (2, 3, 4). Infections with these resistant bacteria are associated with higher mortality rates than those for infections caused by carbapenem-susceptible organisms (5).

The epidemiologic description and phenotypic detection of CRE are complicated by the fact that *Enterobacteriaceae* may be nonsusceptible (intermediate or resistant) to carbapenems via a variety of mechanisms. *Proteus*, *Providencia*, and *Morganella* species demonstrate intrinsically elevated MICs to imipenem (6). *Enterobacteriaceae* can also produce  $\beta$ -lactamase enzymes such as AmpCs (chromosomal or acquired) and ESBLs that do not readily inactivate carbapenems on their own but can confer carbapenem resistance when combined with chromosomal porin mutations that prevent accumulation of  $\beta$ -lactam agents in the bacteria. Finally, the production of carbapenemase enzymes, typically found on mobile genetic elements, that inactivate carbapenem and other  $\beta$ -lactam antibiotics is increasingly common (1, 2). These carbapenemase-producing CRE (CP-CRE) frequently carry multiple resistance mechanisms, which can include redundant  $\beta$ -lactamases such as AmpCs and ESBLs and genes conferring resistance to other antimicrobial classes.

Among the various types of CRE, CP-CRE have received the most attention because they have the greatest potential to contribute to the overall problem of antimicrobial resistance. Production of a carbapenemase usually confers resistance on its own, without requiring additional chromosomal mutations or accessory mechanisms. Because carbapenemase genes are carried on mobile genetic elements, these genes can be spread horizontally to naive bacteria, thus contributing to the reservoir of resistance in both

environmental and clinical *Enterobacteriaceae*. Furthermore, plasmids in CP-CRE often carry additional resistance elements and thus have the potential to increase resistance to multiple drug classes.

$\beta$ -Lactamases are classified by various schemes, the most useful of which describe both their functional and structural profiles (7). Enzymes in groups 1 and 2 (Ambler class C or A and D, respectively) contain an active site serine, but group 1 (primarily AmpC enzymes) members demonstrate preferential hydrolysis of cephalosporins whereas group 2 enzymes (including ESBLs, *Klebsiella pneumoniae* carbapenemase [KPC], and OXA enzymes) are active on a wider range of substrates, extending as broadly as the extended-spectrum cephalosporins and monobactams (ESBL) and the carbapenems (KPC and OXA-48-like). Metallo- $\beta$ -lactamases (MBLs) comprise group 3 (Ambler class B); these include IMP (active on imipenem), VIM (Verona integron-encoded MBL), and NDM (New Delhi MBL), among others. MBLs are characterized by the requirement for zinc ions in their active site, which can be useful diagnostically, as chelators like EDTA inhibit MBL activity by binding zinc. MBLs demonstrate broad-spectrum  $\beta$ -lactamase activity, including carbapenemase activity, but are not active against monobactams. This particular characteristic is not often clinically useful, as most MBL producers also produce other  $\beta$ -lactamases that result in monobactam resistance. CP-CRE enzymes are found in groups 2 and 3, and the diversity of enzyme types contributes to difficulty in both detection and treatment (7).

The epidemiology of CRE varies by country. For example, in the United States, KPC, first identified in a *K. pneumoniae* strain isolated in 1996, is the predominant carbapenemase conferring carbapenem resistance. In some regions of the United States, non-CP-CRE are more common than CP-CRE (8). The prevalence of KPC-producing CRE is unevenly distributed among U.S. states,

Accepted manuscript posted online 6 January 2016

Citation Lutgring JD, Limbago BM. 2016. The problem of carbapenemase-producing-carbapenem-resistant-*Enterobacteriaceae* detection. *J Clin Microbiol* 54:529–534. doi:10.1128/JCM.02771-15.

Editor: C. S. Kraft

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TABLE 1 Characteristics of CRE detection tests for different purposes

Test purpose	Turnaround time	Information needed	Capacity for testing
Therapeutic decision making	Rapid (1 day)	Susceptibility results to guide antimicrobial selection Detect all antimicrobial resistance Avoid overreporting resistance	All clinical microbiology laboratories
Individual facility infection control decisions	Rapid (1–2 days)	Detect all antimicrobial resistance  Detect production of a carbapenemase	Advanced clinical microbiology laboratories Reference laboratories
Epidemiologic research	Less important (can be batched)	Confirmation of facility-level results Identify specific resistance mechanisms	Reference laboratories State or public health laboratories

and the majority of isolates belong to a single species and strain type, *K. pneumoniae* ST258 (9). There are less frequent reports of other carbapenemases in the United States, including NDM, VIM, IMP, and OXA-48-like enzymes (2). The epidemiology is different in other countries. For example, KPC is the most common carbapenemase in Israel, VIM is endemic in Greece, and IMP is endemic in Japan (10). The NDM and OXA-48-like carbapenemases originated in India and Turkey, respectively, where they are endemic, but have successfully disseminated worldwide (11). The regular movement of people colonized or infected with CP-CRE across international borders and the exposure of these people to medical care are an important contributor to the spread of CP-CRE (12).

#### DETECTION OF CARBAPENEMASE-PRODUCING CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE*

Reliable detection of CP-CRE is important for several reasons. Foremost is to guide infection control resources and interventions. Although nearly all CRE are multidrug resistant and therefore generally justify implementation of core infection control measures like hand hygiene and use of contact precautions, many facilities and regions will reserve the most aggressive interventions, such as screening contacts, for CP-CRE (13). CP-CRE possess a more stable and transferable form of resistance with a lower fitness cost than, for example, porin mutations, and this resistance can spread through either clonal expansion or transfer of carbapenemase genes to naive bacteria (9, 11). Following transfer of resistance, newly created CP-CRE can also go on to expand through either mode, thus creating a potential changing target of organisms and resistance profiles. The ability to detect CP-CRE is an important component of outbreak investigations and in the evaluation of potential colonization. Although antimicrobial susceptibility testing results alone are typically required for selection of appropriate therapy, the addition of new antibiotics, like ceftazidime-avibactam, which has activity against some carbapenemases (i.e., KPC) but not others (i.e., NDM), may make detection of specific CP-CRE mechanisms important if these compounds cannot be tested directly (14). Unfortunately, there is already a report of a KPC-producing isolate that is ceftazidime-avibactam resistant. This case illustrates that although knowledge of a specific CP-CRE mechanism may be able to rule out some therapeutic options for patient care, mechanism knowledge cannot reliably predict susceptibility (15). Specific characteristics of various CP-

CRE detection methods may be important to a greater or lesser extent, depending on the situation (Table 1).

The Centers for Disease Control and Prevention (CDC) recently altered its National Healthcare Safety Network (NHSN) surveillance definition for CRE to include *Enterobacteriaceae* (i.e., *Enterobacter*, *Klebsiella*, or *Escherichia coli*) that test as resistant to any of the carbapenem agents, including ertapenem, or demonstrate carbapenemase production through a phenotypic or molecular assay (<http://www.cdc.gov/hai/organisms/cre/definition.html>). The previous definition included *Enterobacteriaceae* that tested as nonsusceptible to imipenem, meropenem, or doripenem and also resistant to all third-generation cephalosporins tested; organisms known to possess intrinsic resistance to imipenem (*Proteus*, *Providencia*, and *Morganella*) were required to be nonsusceptible to a second carbapenem as well. Unfortunately, there is no constellation of phenotypic susceptibility results that will reliably separate CP-CRE from CRE with resistance caused by noncarbapenemase mechanisms. The change to the CDC surveillance definition was made in response to evidence that, based on testing performed in local microbiology laboratories, a definition that excluded ertapenem was not sufficiently sensitive to detect all CP-CRE, including KPC producers. This change was also made despite the recognition that this definition would capture some CRE that were not carbapenemase producers. The former definition and other definitions that do not include ertapenem have the potential to not detect CRE producing OXA-48-like carbapenemases, which are now emerging in the United States (16). One way to refine this definition, and thus improve its specificity for CP-CRE, is to perform a carbapenemase test on all CRE detected. Several methods (both phenotypic and molecular) for CP-CRE are available, as described below and in Table 2.

#### PHENOTYPIC CP-CRE DETECTION METHODS

Once carbapenem resistance is identified through standard susceptibility testing, additional phenotypic tests can help to identify CP-CRE. These include the modified Hodge test (MHT), the Carba NP test and its variants, and the carbapenem inactivation method (CIM). All target carbapenemase production but provide no guidance regarding the specific carbapenemase type. Another option is the use of matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) to detect carbapenemase activity. Like the MHT and the Carba NP test, MALDI-TOF-based assays do not provide insight into which car-

TABLE 2 CP-CRE detection methods

Test method (reference[s])	Accuracy <sup>a</sup>	Turnaround time <sup>b</sup>	Information provided	Limitation(s)	Accessibility <sup>c</sup>
Broth microdilution MBL screen (34)	High	Next day	Detection of MBL	Detects only MBL	Low-moderate (LDT)
Gradient MIC strip (including the Etest KPC and Etest MBL) (33)	Moderate	Next day	Detection of KPC or an MBL	Detects only KPC or MBL; poor specificity when AmpC present	Moderate (commercial RUO)
Multidisc mechanism testing (36, 37)	High	Next day	Detection of KPC, an MBL, or OXA-48	None known	Moderate (commercial RUO, LDT)
Modified Hodge test (17–19)	Moderate	Next day	Detection of carbapenemase activity	Poor sensitivity for NDM producers; poor specificity when AmpC present	High (CLSI- endorsed method)
Carba NP test (including the Rosco Rapid Carb screen, Blue-Carba test, and Rapidec Carba NP test) (18, 23–29)	Moderate	Same day	Detection of carbapenemase activity	Poor sensitivity for OXA-48 producers; poor sensitivity for mucoid isolates	Moderate (CLSI-endorsed method)
Carbapenemase inactivation method (30, 31)	High	Next day	Detection of carbapenemase activity	None known	High (LDT)
MALDI-TOF MS (24, 32)	High	Same day	Detection of carbapenemase activity	None known	Low-moderate (LDT)
PCR, real-time PCR (including LDT, Xpert Carba-R test, hyperplex SuperBug ID, and Check-Direct CPE assay) (38–40)	High	Same day	Detection of specific carbapenemase gene	Unable to detect novel carbapenemase	Low-moderate (CLSI-endorsed method, LDT, commercial RUO)
Microarray (including Verigene, BioFire, and Check-Points) (41, 42, 44)	High	Same day	Detection of specific carbapenemase gene	Unable to detect novel carbapenemase	Low-moderate (FDA approved, commercial RUO)
Whole-genome sequencing (45)	High	Several days	Detection of carbapenem resistance mechanisms	Unable to detect novel carbapenemase	Low (LDT)

<sup>a</sup> Accuracy: high, >90% sensitivity and specificity; moderate, 70 to 90% sensitivity and specificity; low, <70% sensitivity and specificity.

<sup>b</sup> Turnaround time, time to results from pure culture of isolate.

<sup>c</sup> Accessibility: High, all clinical microbiology laboratories could perform this test; moderate, advanced clinical microbiology laboratories could perform this test; low, reference laboratories and/or state or public health laboratories could perform this test.

bapenemase is present, but this assay could gain popularity as MALDI-TOF instruments become more common in clinical laboratories.

The MHT is simple and inexpensive to perform and is well established in many clinical microbiology laboratories in the United States based on its ability to detect KPC producers. The MHT also demonstrates good sensitivity for many other carbapenemases, including VIM, IMP, and OXA-48-like enzymes. For U.S. collections of *Enterobacteriaceae*, sensitivity has been documented between 93 and 98% (17, 18). The test's performance in detection of NDM is generally recognized as much lower; in one study, only 7 of 14 NDM isolates were MHT positive (19). Given the rapid international spread of NDM-producing bacteria, this is an important limitation. In addition, the MHT also suffers from poor specificity because bacteria producing AmpC enzymes combined with porin mutations can give a false-positive result (20, 21, 22). These limitations have resulted in a decrease in the use of MHT outside the United States where KPC is less common than other carbapenemases, but MHT remains an important part of many testing algorithms because it is simple to perform and uses reagents readily available in most microbiology laboratories.

Carba NP is a phenotypic test that detects carbapenemases by measuring the *in vitro* hydrolysis of imipenem by a bacterial extract. Imipenem hydrolysis changes the pH and produces a resul-

tant color change of a pH indicator. The Carba NP is faster than the MHT described above and has excellent sensitivity for most carbapenemases (reported range, 73 to 100%) (18, 23, 24, 25). Its sensitivity for the OXA-48 carbapenemase is much lower; only 1 of 17 OXA-48 producers was detected in one study (24). Similar tests based on the same principle have also been developed, including the Blue-Carba test, the Rosco Rapid Carb screen, and the Rapidec Carba NP test (26, 27, 28, 29).

A newer phenotypic test is the carbapenem inactivation method (CIM) (30, 31). A suspension of the bacterial isolate of interest and water is made, and a meropenem disc is incubated with this suspension. The meropenem disc is then removed and placed on a Mueller-Hinton agar plate that is streaked with a susceptible laboratory strain of *E. coli*. The absence of an inhibition zone indicates hydrolysis of meropenem in the first step and the presence of a carbapenemase. The presence of a clearing zone indicates lack of meropenem hydrolysis (no carbapenemase present) (30). The initial data from this test show a sensitivity of 98 to 100% (30, 31).

MALDI-TOF platforms are well established for rapid bacterial identification, and recently, researchers have been using this tool to detect carbapenemases. MALDI-TOF MS detects specific carbapenem degradation products when bacterial protein extracts are incubated with carbapenems (24, 32). Like the Carba NP test,

MALDI-TOF assays have reported difficulty detecting OXA-48-like enzymes. One study reported a sensitivity of 77% and a specificity of 100%, with the lower sensitivity being due to missing 16 of 19 OXA-48 producers (24). However, this report suggested that the MALDI-TOF carbapenemase assay could be modified (by the addition of bicarbonate) to improve its performance for the OXA-48 producers with little impact on detection of other enzymes (sensitivity improved to 98%) (24). Use of the MALDI-TOF assay for carbapenemase detection is a technique that typically requires the use of alternate MALDI-TOF instrument settings (on the usual instrument) than those used for FDA-approved microbial identification. As such, this test is currently available only to expert users of these systems and requires in-house validation. Its clinical impact is currently negligible, but it may play a role in the future of carbapenemase detection as these instruments become more common and familiar.

More targeted phenotypic carbapenemase assays compare drug activities with and without inhibitors (i.e., EDTA or phenylboronic acid [PBA]) that are specific for given enzyme types (MBL or KPC, respectively). These assays provide evidence of carbapenemase production but also provide information about the type of enzyme produced. For instance, the addition of chelating agents such as EDTA to phenotypic susceptibility tests such as broth microdilution and gradient MIC strips (e.g., Etest) has been used to help confirm the presence of an MBL. Such assays compare the carbapenem MICs obtained with and without chelating agents, which bind zinc ions and therefore inhibit metallo- $\beta$ -lactamase activity. Specific ratios of the standard MIC obtained to that obtained in the presence of the inhibitor are indicative of MBL-type carbapenemases (33, 34). MBL gradient MIC strips have a reported sensitivity of 82 to 94% and a specificity of 97 to 100% (33). Similarly, KPC activity is inhibited by PBA, and thus, PBA has been incorporated into standard antimicrobial susceptibility testing methods for KPC detection. KPC gradient MIC strips have a reported sensitivity of 92% and specificity of 100% (33). However, boronic acid can also inhibit AmpC enzymes, so other testing might be needed to reliably differentiate KPC producers from those with derepressed AmpC enzymes, especially in organisms that carry chromosomal enzymes.

Multidisc diffusion tests involving numerous inhibitors of specific enzyme types, including PBA (inhibitor of KPC and AmpC), EDTA or dipicolinic acid (inhibitors of MBL), clavulanate (inhibitor of ESBL), and cloxacillin (inhibitor of AmpC), have been described for the differentiation of these enzymes from each other, and more recently, a temocillin disc has been added to detect the presence of OXA-48-like carbapenemases (6, 35, 36, 37). The sensitivities of these multidisc tests have ranged between 90 and 100% (36, 37). Such tests are relatively inexpensive and simple to perform, although interpretation, especially when more than one mechanism is present, can sometimes be complicated.

### MOLECULAR CP-CRE DETECTION METHODS

Molecular assays for CP-CRE detection include PCR, microarrays, and whole-genome sequencing (WGS). These methods have the benefit of determining the exact mechanism conferring carbapenem resistance, which can be especially helpful during outbreak investigations and while performing epidemiological research. Unlike the phenotypic assays described above, molecular assays have also been used for detection of common carbapenemase genes directly from stool or rectal swabs and also from

positive blood cultures. The primary limitation of molecular assays is that only known genes can be targeted; those encoding novel carbapenemases will be missed with molecular approaches.

There are multiple PCR assays for detection of carbapenemases, including both laboratory-developed tests (LDT) and commercial assays. Commercial assays include the Xpert Carba-R test (detecting KPC, NDM, IMP, VIM, and OXA-48), the hyperplex SuperBug ID (detecting KPC, NDM, IMP, VIM, GIM, OXA-48, OXA-162, OXA-181, and OXA-204), and the Check-Direct CPE assay (detecting KPC, NDM, VIM, and OXA-48). The sensitivities for all these assays are between 97 and 100% (38, 39, 40). All these commercially developed assays are currently research use only (RUO). PCR allows for rapid identification of specific carbapenemase genes with primers, and probes for real-time assays, to conserved regions in the gene target. PCR is very specific to a given gene and can be further tailored to detect specific subgroups of a gene family. Because PCR assays can be expensive to develop and/or implement, the specific PCR assay to be used would likely depend on the most common resistance genes present in a given geographic region or would target multiple genes simultaneously in a multiplex assay.

Microarray technology utilizes a number of DNA probes that hybridize to DNA targets, including resistance genes. Microarrays can be paired with PCR amplification of target sequences or can be used to directly query DNA in bacterial isolates or patient specimens. The benefit of an array over PCR assays is in the number of targets available for interrogation; while PCR can typically accommodate a maximum of four to five targets per assay, microarrays can include dozens to hundreds of targets, depending on the platform. The capacity inherent in microarrays allows for multiplexing of numerous carbapenemase genes while also being able to distinguish between closely related variants. Several microarray platforms for carbapenemases have been developed, including Verigene, BioFire, and Check-Points. Sensitivities have been reported at 100% (41, 42, 43, 44).

The most comprehensive molecular testing available is whole-genome sequencing (WGS). This technology can query the entire bacterial genome for all known resistance mechanisms and thus can provide resistance information for numerous antimicrobial classes, rather than targeting only carbapenemases, and can identify other contributors to resistance, such as porin mutations. WGS also provides information on the type of plasmid carrying resistance genes, the evolutionary lineage of the bacterium, and the relatedness of isolates, all of which can help to elucidate the source of the isolate or inform outbreak investigations (45). Furthermore, data generated with WGS can be stored for future inquiry as new resistance determinants or virulence factors of interest are identified. Currently, WGS is a rather expensive technology and its use is a fairly specialized process, but as the price drops and analysis pipelines become more automated, WGS is likely to become more widely available.

### SUMMARY

The various CRE and CP-CRE detection methods discussed here address different needs and purposes; there is no one test that is ideal in all situations. When choosing a detection strategy, cost, time to results, test performance (accuracy), and the information provided by the test are all factors that need to be considered. There are many different stakeholders interested in detecting CP-CRE, and this knowledge is useful at the clinical, local, regional,

and national levels. Detection is important for clinicians treating patients with these infections and for infection preventionists and regional CRE prevention collaborations trying to limit the spread of these organisms. As was recently highlighted in the CDC Vital Signs, a coordinated approach to CRE prevention, in which all regional stakeholders are engaged in CRE prevention and informed of CRE prevalence, has the potential to yield significant reductions in CRE transmission compared to traditional, single-institution approaches (46).

## ACKNOWLEDGMENTS

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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