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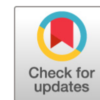
COMMENTARY



CIM City: the Game Continues for a Better Carbapenemase Test

Romney M. Humphries[a,b]

[a]Accelerate Diagnostics, Tucson, Arizona, USA

[b]University of Arizona, Department of Pathology, Tucson, Arizona, USA

ABSTRACT The Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing agree that carbapenemase testing is not necessary for clinical care, provided that the laboratory is up to date with current breakpoints. Nonetheless, publication on the development and modification of carbapenemase tests continues, as is the case in this issue of the *Journal of Clinical Microbiology* (R. W. Beresford and M. Maley, J Clin Microbiol 57:e01852-18, 2019, https://doi.org/10.1128/JCM.01852-18). This commentary explores modifications to the carbapenem inactivation method—but is this the right focus for clinical laboratories?

Among the myriad complex challenges faced by clinical microbiology laboratories, how to best detect carbapenem resistance is one of the foremost. There is no question that carbapenem resistance among Gram-negative bacteria poses a tremendous threat to public health worldwide (1). Carbapenem drugs are the last line of defense against antimicrobial-resistant Gram-negative infections. They are used routinely to treat extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* spp. infections, a practice reinforced by the results of the MERINO trial, which demonstrated significant mortality benefit for the use of meropenem over piperacillin-tazobactam for bacteremia caused by ESBL producers (2). Carbapenems are also a common escalation agent for the treatment of infections caused by nonfermenters, including carbapenem-susceptible isolates of *Pseudomonas aeruginosa* and *Acinetobacter* spp. Infections caused by carbapenem-resistant isolates are associated with mortality rates upwards of 50%, largely due to the lack of treatment alternatives for these isolates (3). The dynamics of the spread of carbapenem resistance involve both transmission of carbapenem-resistant organisms between patients or colonized individuals and transmission of carbapenem resistance determinants on mobile genetic elements between isolates within a single patient (1). Therefore, understanding as soon as possible whether a patient's infection is caused a by carbapenem-resistant isolate is paramount at both the patient and public health levels.

Resistance to the carbapenems is, however, frustratingly complex, and difficult to detect. High-level carbapenem resistance (i.e., a carbapenem MIC above the resistance breakpoint) is only sometimes due to the presence of a carbapenemase. In non-carbapenemase-producing isolates, resistance is due to expression of ESBLs or AmpCs and membrane permeability defects (1); in many institutions, this remains the most common form of carbapenem resistance (3). To further muddy the waters, carbapenemases of clinical significance belong to three distinct molecular classes, including Ambler classes A (e.g., KPC and SME), B (e.g., NDM, IMP, and VIM), and D (OXA-48-like), each of which displays a unique regional epidemiology (1) and challenges to the laboratory (4). New variants within these classes are being described with alarming frequency (5–7).

Recommendations by laboratory standards development organizations (SDOs) like

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Address correspondence to rhumphries@axdx.com.

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TABLE 1 Evolution of CLSI carbapenemase testing recommendations

Time period or year	CLSI recommendations in M100
Pre-2000	No specific recommendations regarding carbapenem resistance testing
2000s	Recognition of the threat of KPC-expressing *K. pneumoniae* Recognition that carbapenem breakpoints were unlikely to predict clinical outcomes for isolates with elevated carbapenem MICs
2009	Introduction of modified Hodge test (MHT) for isolates of *Enterobacteriaceae* with elevated carbapenem MICs If MHT is positive, laboratories instructed to edit all carbapenem results to "R"
2010	*Enterobacteriaceae* carbapenem breakpoints revised Change of recommendation for MHT to "optional" for infection control/epidemiology
2012	*Pseudomonas aeruginosa* carbapenem breakpoints revised Carba NP test introduced for *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.
2014	*Acinetobacter* spp. carbapenem breakpoints revised
2017	mCIM for *Enterobacteriaceae* introduced
2018	MHT eliminated eCIM introduced for *Enterobacteriaceae* Carba NP for *Acinetobacter* spp. removed mCIM for *P. aeruginosa* introduced

the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are continually evolving to meet these challenges. Since 2009, when the modified Hodge test (MHT) was first introduced by CLSI to address the spread of KPC-expressing *Klebsiella pneumoniae*, CLSI carbapenemase testing recommendations have been a shifting target (Table 1). Against this backdrop is the continued debate over the ultimate purpose(s) for testing clinical isolates for carbapenemases: clinical decision making, infection control practices, and/or epidemiological studies. Experts remains divided on this point (8), but CLSI and EUCAST agree that carbapenemase testing is not necessary if the laboratory is up to date with current breakpoints.

The complexity of these important challenges is reflected in the interest of the clinical microbiology community regarding carbapenemase testing. A review of the *Journal of Clinical Microbiology* finds 16 articles posted on this topic since 2018 alone. Many of these studies report "improvements" over previously reported methods. As an example, 10 variations of the carbapenem inactivation method (CIM), first described in 2015 (9), have been published, and are listed in Table 2. The CIM is an attractive option for carbapenemase testing by clinical laboratories, as the method can be performed using reagents readily at hand in most laboratories and testing does not require special equipment. The CLSI specifically endorsed a modification to the CIM (mCIM), which included use of an alternative incubation medium (tryptic soy broth versus water) and extension of the incubation time to 4 h, both of which were found to improve sensitivity for certain carbapenemases (10). CLSI followed this with a modification for use with *P. aeruginosa* isolates (11) and, most recently, with recommendations to add EDTA to the mCIM (eCIM), which allows differentiation of class B from class A and D carbapenemases (Table 2) (12). The eCIM must be used in conjunction with the mCIM, as both carbapenem inactivation and inhibition of the inactivation by EDTA for a class B enzyme must be demonstrated. It should be noted that isolates that express both a class A and/or D and class B carbapenemase, which are increasingly common, may give false-negative results by the eCIM for a class B carbapenemase (12). In this issue of the *Journal of Clinical Microbiology,* Beresford and Maley (13) further describe a modification

TABLE 2 Variations of the CIM reported in the literature

Method	Description[a]	Organism(s)	Reference
CIM	Harvest 10-ul loop of isolate from Mueller-Hinton or BAP Suspend isolate in 400 μl water Add 10-μg meropenem disk to suspension Incubate 2 h at 35°C Remove disk, use for standard disk diffusion of *E. coli* 25922 Incubate plate for 6 h or overnight Evaluate zone of inhibition around disk: No zone = carbapenemase present Zone of inhibition = no carbapenemase	*Enterobacteriaceae*	9
Modified CIM (mCIM)	Increased sensitivity for OXA-48-like producers Suspend 1 μl of isolate in TSB Incubate 4 h at 35°C Incubate disk diffusion plate a full 18 h	*Enterobacteriaceae*	10
mCIM for *P. aeruginosa*	Increased sensitivity for *P. aeruginosa* Increased inoculum (10 μl) vs mCIM	*P. aeruginosa*	11
CIMPlus	Addition of inhibitors (EDTA, phenylboronic acid) to water to differentiate carbapenemase Ambler Classes	*Enterobacteriaceae*	22
Simplified CIM (sCIM)	Improved simplicity Use of 10-μg imipenem disk Isolate smeared directly onto disk from BAP No incubation of disk and test isolate prior to use in disk diffusion test	*Enterobacteriaceae*, *P. aeruginosa*	23
Triton X CIM (TCIM)	Improved performance for *Acinetobacter* spp. Cell permeabilized by adding 0.1% (vol/vol) Triton X-100 to TSB Use of 10 μl of test isolate	*A. baumannii*	24
CIMTris	Improved detection in *Acinetobacter* and *Pseudomonas* spp. Use of Tris-HCl buffer to extract carbapenemase from cell	*A. baumannii*, *P. aeruginosa*	25
CIMTrisII	Improved detection in *A. baumannii* and *P. aeruginosa* 5-μg meropenem disk 5-μl loop of bacteria Use of Tris-HCl buffer to extract carbapenemase Incubation time, 1 h	*A. baumannii*, *P. aeruginosa*, *Enterobacteriaceae*	26
Rapid CIM (rCIM)	More rapid detection (~2.5 h) of carbapenemase: Use of 20 μl of overnight culture Homogenize in 1 ml sterile water Add 2 10-μg meropenem disks Incubate 30 min at 37C Harvest cells by centrifugation Add and mix 500 μl of supernatant with 2.5 ml of a 1 McFarland suspension of *E. coli* ATCC 25922 in TSB Incubate at 37°C for 1.5–2 h Evaluate growth of *E. coli* using a nephelometer	*Enterobacteriaceae*	27
EDTA CIM (eCIM)	Differentiation of class B carbapenemases Addition of EDTA to TSB for mCIM	*Enterobacteriaceae*	12
Automated CIM	Plates are imaged on a BD Kiestra Work Cell incubator and zone diameters evaluated using the ReadA program	*Enterobacteriaceae*	13

[a]BAP, blood agar plate.

of the mCIM, through the use of digital microbiology to automate test reading and shorten the incubation time.

What is interesting about the studies listed in Table 2 is that each reported excellent (>95%) sensitivity and specificity for carbapenemase detection, with the exception of test methods for *Acinetobacter* spp. Why, then, are we compelled to continue to gild the lily? The answer seems to stem from not only the complex spectrum of enzymes associated with carbapenem resistance but the differing spectrum of these encoun-

tered in laboratories globally and the varied practices to which the results of carbapenemase testing are applied. Thus, each laboratory may need to adapt the method to ensure that (i) carbapenemases prevalent in the laboratory's region are detected; (ii) results are easy to interpret, with a low prevalence of false-positives; (iii) different carbapenemase classes are differentiated, if needed for clinical care/infection control; and (iv) results are reported in a time frame that best supports implementation of contact precautions and/or treatment changes. Each of these requirements highlights the necessity for the laboratory to carefully verify carbapenemase tests prior to implementation. Beresford and Maley carefully vetted their modified mCIM using a collection of IMP-producing isolates, a resistance mechanism rare to most parts of the world but endemic to Australia (13). The authors found that a minimum of 12 h of incubation time was needed to ensure detection of these weak carbapenem hydrolyzers. The authors are careful to point out that these modifications work for isolates that display significant elevations to carbapenem MICs but may not detect those that harbor bla_{IMP} and are carbapenem susceptible. As the authors further discuss, low specificity and/or high frequency of indeterminate results are equally important when evaluating carbapenemase testing options. Labeling a patient as positive for a carbapenemase-producing organism may lead to long-term infection control repercussions, such as life-long, preemptive contact isolation, and/or use of suboptimal therapeutic options, such as colistin, in regions where newer antimicrobials with activity against class A and D carbapenemase producers are not available.

Given this complex and changing picture of carbapenemase testing, it is perhaps no surprise to find that laboratory carbapenemase testing practices in both the United States (as documented in California) and Europe vary considerably (14, 15). Surveys demonstrate a shocking number of laboratories that continue to apply the MHT (57% in Europe and84% in California [14, 15]), a method that is fraught with both false-positive and false-negative results (4) and that is no longer recommended by CLSI or EUCAST. This demonstrates that many laboratories are not able to adapt to the changing landscape of carbapenemase epidemiology, which may occur over a very short time frame within a single institution (16). Given this context, laboratories are best served to ensure that the cornerstone for carbapenem resistance testing is use of up-to-date breakpoints, as these detect clinically significant resistance. Unfortunately, roughly 30% of California laboratories continued to apply 2009 carbapenem breakpoints (15). Carbapenem resistance does not silo in individual hospitals (17, 18), and computer model estimates demonstrate that interrupting the spread of carbapenem resistance requires a coordinated regional response (19, 20). This challenge requires active participation of diagnostic manufacturers, the U.S. Food and Drug Administration, clinical laboratories, public health authorities, laboratory accreditation organizations, and hospital administrators. Pilot programs implemented by the CDC's Antimicrobial Resistance Laboratory Network and independent activity by local public health jurisdictions to address these challenges demonstrate that change is possible (21). If we can learn anything from (SIM) city planning, focusing all development efforts on dense commercial activities while neglecting residential needs never wins the game.

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