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Is this the carbapenemase test we've been waiting for? A multi-center evaluation of the Modified Carbapenem Inactivation Method (mCIM).

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Abstract

A plethora of phenotypic methods exist for the detection of carbapenemases, however, clinical laboratories have struggled for years with accurate, objective phenotypic detection of carbapenemase activity in *Enterobacteriaceae*. In this issue of the *Journal of Clinical Microbiology*, V. M. Pierce et al. (J Clin Microbiol 55:XXX-XXX, 2017, https://doi.org/10.1128/JCM.00193-17) report on a multicenter evaluation of the modified carbapenem inactivation method (mCIM). The high sensitivity, specificity, reproducibility and ease of interpretation associated with mCIM for *Enterobacteriaceae* will likely lead to its adoption by clinical laboratories.

Text

Carbapenems are considered the treatment of choice for serious infections caused by multi-drug resistant *Enterobacteriaceae*. However, the emergence and successful global dissemination of carbapenemase-producing *Enterobacteriaceae* presents a clinical challenge and is a growing public health emergency (1). The prevailing perception that these organisms are the next "nightmare bacteria" conjures images of the pre-antibiotic era where even the most common of infections could prove fatal. No longer are these strains confined to the hospital environment, but they are now found in the community, environment, livestock, companion animals, and wildlife (2, 3). Rapid, accurate detection of the infecting culprit is a central tenet to tackling the spread of any infectious disease and this is also true for carbapenem-resistant

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Enterobacteriaceae (CRE) where the mortality rate for serious infections exceeds 40% in patients with severe underlying conditions (4).

The CRE story is rife with controversy and challenges. Notably, establishment of a common global definition has been problematic. The CDC definition of carbapenem-resistant *Enterobacteriaceae* (CRE) has evolved to encompass isolates that test resistant to any carbapenem tested, as well as any isolate found to harbor a carbapenemase either by detection of the gene or phenotypic detection of carbapenemase activity. This definition shows improved sensitivity compared with the previously used definition which misclassified 21% of KPC-producing *K. pneumoniae* as non-CRE (5). Importantly, this definition is not specific to carbapenemase-production and carbapenem resistance in *Enterobacteriaceae* encompasses organisms that produce a carbapenemase (so-called carbapenemase-producing or CP-CRE) as well as those that do not (i.e. non-CP-CRE). Non-CP-mediated resistance most commonly occurs due to the expression of an extended-spectrum or an AmpC beta-lactamase combined with a permeability defect such as porin loss. It is believed that the non-CP-CRE may be associated with a fitness cost to the organism and consequently may not disseminate as effectively as their CP-CRE brethren, although outbreaks of non-CP-CRE do occur (6). Because carbapenemase genes are harbored on mobile DNA elements and can be easily spread, the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommend that laboratories continue to look for

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carbapenemases for epidemiology and infection prevention purposes but that detection is not necessarily pertinent to therapeutic decision-making.

The concept that minimum inhibitory concentration (MIC) rather than the presence of carbapenemase should be used to guide clinical treatment decisions has been controversial since initially proposed by CLSI and EUCAST in 2010 (7). Revised lower breakpoints enable clinical laboratories to better detect carbapenemase-producing organisms as part of routine susceptibility testing without having to rely on confirmatory phenotypic detection of carbapenemase activity. The revision in 2010 was supported by outcome data, albeit relatively limited, that MIC correlates better with patient outcome rather than the presence of a carbapenemase per se (8). Additionally, pharmacokinetic/pharmacodynamics studies suggest that carbapenems can successfully be used to treat CRE with MICs up to 4µg/mL, or even as high as 8µg/mL, using optimized dosing strategies such as high-dose, prolonged infusion, and combination regimens (9). Consequently, it is no longer recommended to perform testing for carbapenemases for susceptibility testing purposes nor is it recommended to modify the interpretation of carbapenem susceptibility testing if a carbapenemase is detected.

At present, there remains a dearth of outcome data either supporting or disputing the current susceptibility reporting guidelines. Furthermore, in the absence of published prospective randomized controlled trials, recommended therapy for the treatment of serious infections caused by carbapenemase-producing *Enterobacteriaceae* is based primarily on observational studies. Data

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92 are available to support the use of combination therapy for the treatment of
93 serious infections (10, 11); however, these benefits may primarily be limited to
94 those patients with severe underlying conditions (12). Collectively, inclusion of a
95 carbapenem in the treatment of carbapenemase-producing *Enterobacteriaceae*
96 appears to be associated with lower mortality rates compared to carbapenem-
97 sparing treatment regimens, but this has not borne out in all observational
98 studies (13). Without unequivocal outcome data supporting the 2010 CLSI and
99 EUCAST guidelines, physicians may be reluctant to treat CP-CRE with a
100 susceptible carbapenem.

101 Although the most commonly encountered carbapenemases in the United
102 States are variants of the *Klebsiella pneumoniae* carbapenemase (KPC), the
103 epidemiology of carbapenemase-producing *Enterobacteriaceae* varies
104 geographically (14) and outbreaks due to other carbapenemases have also
105 occurred in this country e.g. duodenoscope-related outbreaks of infection caused
106 by New Dehli metallo-beta-lactamase (NDM)-producing *E. coli* (15) and
107 oxacillinase (OXA)-232-producing *K. pneumoniae* (16). The availability of an
108 accurate, cheap and easy-to-perform method capable of detecting all major
109 carbapenemase families for routine use in clinical microbiology laboratories has
110 been elusive. The modified Hodge test demonstrates inferior sensitivity for the
111 detection of NDM, lacks appropriate specificity, is highly subjective, and will be
112 removed from the CLSI M100S Edition Standard beginning next year. The
113 Carba-NP assay (CLSI M100S), which colorimetrically detects the pH change
114 that occurs upon hydrolysis of imipenem, shows high sensitivity for many

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carbapenemases though the detection of OXA-48 carbapenemases is problematic. Even though the Carba-NP assay produces results in less than two hours, interpretation of the assay is subjective and daily reagent preparation is time-consuming, although commercially available tests employing this method are available. While there are FDA-cleared assays available for the molecular detection of major carbapenemase families, use of such assays may be costly for laboratories in areas of high CP-CRE prevalence. Other methods have also been used to detect carbapenemase production (e.g. MALDI-TOF, inhibitor tests) and these methods also have their own limitations. The interested reader is referred to the following excellent recent reviews for a more detailed description of carbapenemase detection methods (17, 18).

In this issue of the *Journal of Clinical Microbiology*, Pierce and colleagues describe a multi-center evaluation of a modified carbapenem inactivation method assay (mCIM) (19). The original carbapenem inactivation method (CIM), first published by van der Zwaluw and colleagues, is based on a simple principle using reagents readily available to clinical microbiology laboratories (20). Namely, that the contents of a 10µg meropenem disk are degraded through enzymatic activity when the disk is incubated in a bacterial suspension of carbapenem-producing organism. Meropenem degradation is assessed by subsequently incubating the disk on a lawn of a sensitive *E. coli* indicator strain. In the original description of the assay, the authors reported 100% correlation with carbapenemase gene PCR. However, subsequent studies show reduced

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sensitivity of the CIM assay for the detection of both OXA-48 and NDM carbapenemases (21, 22).

To improve sensitivity for the detection of carbapenemases that be expressed at low levels or that naturally have weaker hydrolytic activities, the mCIM assay uses less organism (1µL vs. 10µL loopful), a longer incubation period of the disk-bacterial suspension (4 vs. 2 hours), and a different suspension matrix (tryptic soy broth vs. water) than the original CIM assay. Importantly, Pierce et al ascribe and validate diffusion cut-offs that include an "indeterminate" interpretive criteria. This is the first multi-center evaluation of the mCIM method and the only publication to evaluate the reproducibility of the assay. The study was performed across nine distinct centers in the United States using isolates that had been fully characterized by whole genome sequencing or PCR. This approach enabled the authors to assess performance of the assay for a broad balance of different carbapenemase families while avoiding potential performance bias from use of prospective clinical strains that may be clonal.

Pierce and colleagues found the mCIM assay to have excellent sensitivity and specificity: 23 of 27 known carbapenemase-producing *Enterobacteriaceae* isolates were mCIM assay-positive in all nine sites with the three additional isolates positive in 8 of the 9 laboratories. Similar results were observed for the 34 isolates known to be negative for carbapenemases. Overall, sensitivity from the multi-center evaluation was 93-100% with a mean sensitivity with a mean specificity of 99% (range: 97-100%). Importantly, the performance of the mCIM and CIM assays were directly compared at one of the test sites. Using 115 fully-

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characterized *Enterobacteriaceae* isolates, the mCIM assay demonstrated improved sensitivity for the detection of carbapenemase-producing *Enterobacteriaceae* compared to the original CIM assay (94% vs. 82%) while the specificity for both assays was 100%. Importantly, the results of this multi-center evaluation have led to incorporation of the mCIM method into 27th edition of the CLSI M100S document.

As mentioned previously, CLSI and EUCAST no longer recommend that clinical laboratories test for carbapenemase for treatment purposes. However, one recently published study suggests that this approach may need rethinking. In a recent study by Tamma et al looking at 83 unique episodes of monomicrobial bacteremia due to CRE, the authors observed a more than 4 times greater odds of dying within 14 days when adjusted for severity of illness of the first day of bacteremia (23). Alarmingly, differences in mortality between CP and non-CP-CRE were still observed when the authors limited their analysis to strains with meropenem MIC's ≤4µg/mL. Prior to this publication, no studies had adequately assessed whether there are observable differences in clinical outcomes between CP and non-CP CRE. It is important to note that 92% of the carbapenemase-producing organisms in this study harbored a KPC and thus whether these findings extend to other carbapenemase families remains to be seen.

Dectection of carbapenemase activity is undeniably important for epidemiological and infection control purposes and many would argue that mechanistic inforamtion is beneficial when making treatment decisions. These data therefore highlight the need for laboratories to have ready access to a

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reliable method for carbapenemase detection. As described by Pierce et al, the mCIM assay appears to fit that bill for *Enterobacteriaceae*. Nevertheless, preliminary reports to assess performance using non- *Enterobacteriaceae* isolates have revealed the current version of the mCIM assay may not be the one-size fits all carbapenemase test that laboratories have been longing for. Compared to other available methods, the mCIM appears to offer clinical laboratories an affordable assay using materials and methods that are familiar, all while having a test that is interpreted objectively. Whether the assay can reliably detect other carbapenemase families (e.g. GES) or whether shorter incubation periods can be used deserves further study.

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