Determining the Optimal Carbapenem MIC That Distinguishes Carbapenemase-Producing and Non-Carbapenemase-Producing Carbapenem-Resistant *Enterobacteriaceae*

Pranita D. Tamma,^a Yanjie Huang,^b Belita N. A. Opene,^c Patricia J. Simner^c

Division of Pediatric Infectious Disease, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA^a; Department of Health Policy and Management, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA^b; Division of Medical Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA^c

Carbapenemase-producing (CP) *Enterobacteriaceae* are largely responsible for the rapid spread of carbapenem-resistant *Enterobacteriaceae* (CRE). Distinguishing CP-CRE from non-CP-CRE has important infection control implications. In a cohort of 198 CRE isolates, for isolates that remained susceptible or intermediate to some carbapenem antibiotics, an ertapenem MIC of 0.5 µg/ml and meropenem, imipenem, and doripenem MICs of 2 µg/ml were best able to distinguish CP-CRE from non-CP-CRE isolates.

Carbapenem-resistant *Enterobacteriaceae* (CRE) encompass both carbapenemase-producing (CP) and non-CP types. CP-CRE are particularly concerning from an infection control standpoint, as the genes encoding carbapenemases are generally located on mobile genetic elements (i.e., plasmids, transposons, and insertion sequences) and are easily transmissible to other Gram-negative organisms (1). In the United States, carbapenemase genes are most commonly *bla*_{KPC} (2). However, other carbapenemase genes (e.g., *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{OXA}) have been increasingly encountered, due in large part to international migration and medical tourism (3). Gram-negative organisms harboring these resistance genes can spread easily from patient to patient in health care settings. In fact, CP-CRE have been responsible for a number of outbreaks in the health care environment (1). On the contrary, non-CP-CRE generally emerge as a result of heterogeneous mechanisms, such as reduced outer membrane permeability, and have been associated with a loss of organism fitness and reduced transmissibility (1). Distinguishing among these resistance mechanisms has important infection control implications.

The Centers for Disease Control and Prevention (CDC) acknowledges that “CP-CRE are currently believed to be primarily responsible for the increasing spread of CRE in the United States and have therefore been targeted for aggressive prevention,” and that “a reliable way to differentiate CP-CRE from non-CP-CRE might help guide such targeting by identifying the organisms of greatest epidemiological interest” (4). Determining whether a CRE is producing carbapenemases involves several additional steps in the microbiology laboratory and can be resource intensive. As a result, many US clinical microbiology laboratories do not conduct additional phenotypic tests, such as the Carba NP test or modified Hodge test to determine if CRE are indeed CP-CRE (2). These tests have limitations in their ability to detect some CP genes (e.g., the Carba NP is limited in detection of *bla*_{OXA-48-type}, and the modified Hodge test is limited in detecting metallo-β-lactamases) (5, 6).

Currently, most health care facilities follow CDC recommendations to institute contact precautions when patients with CRE are identified and subsequently perform unit-wide point prevalence studies to identify other unrecognized carriers on the unit (7). We sought to determine whether an optimal carbapenem MIC can reliably distinguish CP-CRE from non-CP-CRE, thus

potentially lessening the burden on microbiology laboratories and infection preventionists.

We included well-characterized CRE isolates ($n = 145$) from the Antimicrobial Resistance Isolate Bank obtained between 2012 and 2015 (8) and all CRE bloodstream isolates from unique patients at The Johns Hopkins Hospital between 2014 and 2015 ($n = 53$) (Table 1). The Antimicrobial Resistance Isolate Bank is a repository of bacteria with genotypic and susceptibility data that have been assembled by the CDC and Food and Drug Administration. For The Johns Hopkins Hospital isolates, carbapenem MICs were determined using the Etest (bioMérieux). Carbapenem resistance was defined according to the Clinical and Laboratory Standards Institute (CLSI) criteria, with ertapenem resistance defined as an MIC of ≥ 2 µg/ml, and meropenem, imipenem, and doripenem resistance defined as an MIC of ≥ 4 µg/ml (9). β-Lactamase gene identification was performed using the Check-MDR CT103XL assay (Check-Points, Wageningen, The Netherlands), which combines PCR amplification and microarray technologies for the detection of an extended panel of carbapenemase genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{OXA-48-like}, *bla*_{GES}, *bla*_{GIM}, *bla*_{SPM}, *bla*_{OXA-23-like}, *bla*_{OXA-24/40-like}, and *bla*_{OXA-58-like}) (10).

We created histograms to distinguish the MIC ranges of CP-CRE and non-CP-CRE individually for ertapenem, meropenem, imipenem, and doripenem. The Wilcoxon rank sum test was used to compare medians and interquartile ranges (IQR) between carbapenem MICs for CP-CRE and non-CP-CRE isolates for each of the antibiotics, with a two-tailed P value of <0.05 considered statistically significant. A receiver operating characteristic (ROC) curve was generated using var-

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Address correspondence to Pranita D. Tamma, ptamma1@jhmi.edu.

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TABLE 1 Bacterial genus and species and β-lactamase genes identified in a cohort of 198 carbapenem-resistant Enterobacteriaceae isolates

No. of each bacterial species (n)																	
β-Lactamase class	Type of β-lactamase	<i>Citrobacter amalonuticus</i> (1)	<i>Citrobacter freundii</i> (2)	<i>Enterobacter aerogenes</i> (7)	<i>Enterobacter asburiae</i> (1)	<i>Enterobacter cloacae</i> (40)	<i>Escherichia coli</i> (30)	<i>Klebsiella oxytoca</i> (4)	<i>Klebsiella ozonae</i> (2)	<i>Klebsiella pneumoniae</i> (96)	<i>Morganella morganii</i> (2)	<i>Proteus mirabilis</i> (3)	<i>Providencia rettgeri</i> (1)	<i>Raoultella</i> spp. (1)	<i>Salmonella</i> spp. (1)	<i>Serratia marcescens</i> (7)	
Class A																	
TEM (8) TEM, ACT/MIR (3) SHV (4) SHV, ACT/MIR (3) TEM, CTX-M, SHV (6) CTX-M, SHV (4) CTX-M (5) CTX-M, DHA (3) TEM, SHV (4) TEM, CTX-M, SHV (1) KPC (29) KPC, TEM, CTX-M, SHV (6) KPC, CTX-M, SHV (3) KPC, SHV, TEM (5) KPC, ACT/MIR (1) KPC, CTX-M, ACT/MIR (1) KPC, ACT/MIR, TEM (1) KPC, TEM (4) KPC, SHV (8) IMI (2) SME (7)	ESBL ^a	1				4	3										
	ESBL, AmpC					3											
	ESBL							2		2							
	ESBL, AmpC					3											
	ESBL									6							
	ESBL									4							
	ESBL						1			4							
	ESBL, AmpC									4							
	ESBL					4					3						
	ESBL										1						
	Carbapenemase						6	3	2	1	12	1	2		1		
	ESBL, carbapenemase										6						
	ESBL, carbapenemase										3						
	ESBL, carbapenemase						2				3						
	AmpC, carbapenemase					1											
ESBL, AmpC, carbapenemase						1											
ESBL, carbapenemase										1							
ESBL, carbapenemase						2	2										
ESBL, carbapenemase						2				8							
Carbapenemase																	
Carbapenemase																	
Class B																	
NDM (28) NDM, CTX-M (4) VIM (8) VIM, CMY (1) IMP (4) NDM, TEM, CTX-M, SHV (3) NDM, DHA, CTX-M, SHV (3)	Carbapenemase	1				1	10			12	1	1	1		1		
	ESBL, carbapenemase						4										
	Carbapenemase					2				6							
	ESBL, AmpC, carbapenemase									1							
	Carbapenemase									2							
	Carbapenemase									2							
	ESBL, carbapenemase									3							
	ESBL, carbapenemase									3							
	ESBL, AmpC, carbapenemase									3							
	Class C																
cAmpC (8) CMY (7) ACT/MIR (7)	AmpC			2		6											
	AmpC						7										
	AmpC					4				3							
Class D																	
OXA-48 (7) OXA-232 (2) OXA-181 (7) OXA-48, CTX-M, SHV, TEM (1)	Carbapenemase			3						4							
	Carbapenemase									2							
	Carbapenemase								1	6							
	Carbapenemase									1							
	ESBL, carbapenemase										1						

^a ESBL, extended-spectrum β-lactamase.

FIG 1 Distributions of carbapenem-resistant *Enterobacteriaceae* carbapenem MICs against carbapenemase producers and non-carbapenemase producers: (A) ertapenem, (B) meropenem, (C) imipenem, and (D) doripenem. Gray bars represent non-carbapenemase producers, and black bars represent carbapenemase producers.

ious carbapenem MICs to determine the optimal MIC for the detection of CP-CRE. The discriminatory power was evaluated using the area under the ROC curve (AUC), with an AUC value of 0.5 indicating no discriminative ability and an AUC of >0.8 indicating good to excellent prediction. The sensitivity and specificity of the prediction rule were calculated at various carbapenem MIC values. Statistical analyses were performed using the R statistical package.

There were 135 (68%) CP-CRE and 63 (32%) non-CP-CRE isolates. The genus and species of the isolates in the full cohort are displayed in Table 1. For the 53 Johns Hopkins Hospital isolates, the most commonly recovered organisms were *Klebsiella pneumoniae* (53%), *Enterobacter* spp. (42%), and *Escherichia coli* (5%). An evaluation of the full cohort revealed that the CP-CRE isolates contained a variety of serine and metallo- β -lactamase carbapenemase genes encoding class A carbapenemases (*bla*_{KPC} [$n = 58$], *bla*_{SME} [$n = 7$], and *bla*_{IMI} [$n = 2$]), class B carbapenemases (*bla*_{NDM} [$n = 38$], *bla*_{IMP} [$n = 4$], *bla*_{VIM} [$n = 9$]), and class D carbapenemases (*bla*_{OXA-48-type} [$n = 17$]) (Table 1). Twenty-three (43%) of the Johns Hopkins Hospital isolates were CP-CRE, including 21 (92%) *bla*_{KPC}, 1 (4%) *bla*_{NDM}, and 1 (4%) *bla*_{OXA-48-type}.

The distribution of MICs for each of the carbapenems is displayed in Fig. 1. The carbapenems tested (and their median and IQR MICs against CP-CRE) were as follows: ertapenem (16 μ g/ml [8 to 16 μ g/ml]), meropenem (16 μ g/ml [4 to 16 μ g/ml]), imipenem (16 μ g/ml [4 to 64 μ g/ml]), and doripenem (16 μ g/ml [4 to 16 μ g/ml]). The carbapenems tested (and their median and IQR MICs against non-CP-CRE) were as follows: ertapenem (2 μ g/ml [0.125 to 16 μ g/ml]), meropenem (1 μ g/ml [1 to 2 μ g/ml]), imipenem (1 μ g/ml [1 to 2 μ g/ml]), and doripenem (1 μ g/ml [0.5 to 2 μ g/ml]). For each of these antibiotics, the distributions of carbapenem MICs for CP-CRE and non-CP-CRE were significantly different ($P < 0.01$). No differences in the carbapenem MIC distributions were noted between isolates producing the carbapenemase genes most frequently identified in samples from the United States (*bla*_{KPC}, *bla*_{NDM}, and *bla*_{OXA-48-like}); however, there were small numbers of carbapenemases other than *bla*_{KPC} and *bla*_{NDM} in our cohort, which limited a robust comparison of the MIC distributions for the different carbapenemases.

We developed an ROC curve for each of the carbapenem antibiotics to identify the carbapenem MIC that best distinguishes CP-CRE from non-CP-CRE (Fig. 2). For ertapenem, a group 1

FIG 2 Receiver operating characteristic curves using carbapenem MICs for the detection of carbapenemase-producing carbapenem-resistant *Enterobacteriaceae*: (A) ertapenem, (B) meropenem, (C) imipenem, and (D) doripenem.

carbapenem, the AUC of the ROC curve was 0.79. An MIC cutoff of 0.5 $\mu\text{g/ml}$ had the greatest overall sensitivity and specificity (98.2% and 61.1%, respectively) for distinguishing CP-CRE from non-CP-CRE. The AUCs were >0.90 for the group 2 carbapenems meropenem, imipenem, and doripenem, with sensitivities ranging from 89.5% to 94.5% and specificities ranging from 77.8% to 83.3%, indicating that these agents had an excellent ability to distinguish CP-CRE from non-CP-CRE at an MIC of 2 $\mu\text{g/ml}$.

The use of an MIC cutoff to distinguish CP-CRE from non-CP-CRE, if one exists, might reduce the burden on clinical microbiology laboratories when deciding to conduct point prevalence surveillance studies in the case that a patient with a CRE infection is identified. Additionally, a reliable MIC cutoff might avoid the need for the additional steps involved in implementing phenotypic methods to distinguish CP from non-CP-CRE. Our findings suggest that if a CRE isolate remained susceptible or intermediate to some carbapenems, an ertapenem MIC of 0.5 $\mu\text{g/ml}$ or greater

or group 2 carbapenem MICs of 2 $\mu\text{g/ml}$ or greater represent the carbapenem MICs that are best able to discriminate between CP and non-CP-CRE. Based on the carbapenem ROC curve we generated, the specificity of the MIC cutoff for ertapenem and the group 2 carbapenems is only approximately 60% and 80%, respectively. The excellent sensitivity, albeit less than ideal specificity, of these carbapenem MIC cutoffs would ensure that almost all patients with a CP-CRE infection could be rapidly identified and placed on contact precautions, potentially limiting their spread in healthcare institutions. Because of the significant morbidity and mortality rates associated with CP-CRE infection, we favor approaches to optimizing the sensitivity of CP-CRE detection, even if they require some compromise in specificity.

There are limitations with our study. We realize that susceptibility patterns might change over time and that these data need to be reviewed periodically to remain accurate. Additionally, we realize that the assortment of carbapenemases and proportion of CP-CRE and non-CP-CRE isolates may not be generalizable to

other clinical settings, and these data will vary on the basis of local epidemiology. In our cohort, the majority of carbapenemases were *bla*_{KPC}, followed by *bla*_{NDM}. While this is consistent with what others have observed in the United States (2), it differs from carbapenemase distributions observed globally (3), and it is not known if our findings would be replicated in settings outside the United States.

Nonetheless, until rapid, accurate, cost-effective, and standardized methods of carbapenemase detection are available in clinical microbiology laboratories across the United States, we believe that alternate approaches might be useful for differentiating CP-CRE and non-CP-CRE infection. We encourage other research groups to repeat these analyses with larger cohorts to increase the accuracy of carbapenem MICs for distinguishing CP-CRE from non-CP-CRE.

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