Use of Ancillary Carbapenemase Tests To Improve Specificity of Phenotypic Definitions for Carbapenemase-Producing *Enterobacteriaceae*

Shelley A. Miller, Janet A. Hindler, Angelo Chengcuenca, Romney M. Humphries

Department of Pathology & Laboratory Medicine, UCLA, Los Angeles, California, USA

ABSTRACT Carbapenemase-producing *Enterobacteriaceae* (CPE) are a significant threat to public health. In 2015, CDC revised the surveillance definition for CPE to include all *Enterobacteriaceae* resistant to any carbapenem tested. However, this definition is associated with poor specificity. We evaluated the performance of this definition, compared to carbapenemase PCR, for a collection of 125 *Enterobacteriaceae*. We also investigated the impact of ancillary testing for carbapenemase of isolates that met the CDC CPE surveillance definition. The two ancillary tests evaluated were the Xpert Carba-R assay, a molecular test, and the carbapenem inactivation method (CIM). Two variables were evaluated for the CIM: suspension of organisms in double-distilled water (ddH₂O) versus tryptic soy broth (TSB) to incubate disks, and incubation of plates for 6 h versus 18 to 20 h. The sensitivity and specificity of the Carba-R assay were 100% compared to the results of in-house PCR. The sensitivities of the CIM performed with TSB were 94.6% when read at 6 h and 97.7% when read at 18 to 20 h; the sensitivities with ddH₂O were 88.0% when read at 6 h and 93.0% when incubated for 18 to 20 h. The specificity was 100% for all variables tested. Without ancillary testing, the sensitivity of the CDC definition was 98.9% for CPE, and the specificity was 6.1%. Testing isolates that screened positive by the CDC definition with the Xpert Carba-R did not change the sensitivity, and it improved the specificity to 100%. Similarly, the use of the CIM (TSB and 18 to 20 h of incubation) to confirm screen-positive isolates resulted in a sensitivity of 95.6% and specificity of 100%.

KEYWORDS Carba-R, *Enterobacteriaceae*, carbapenemase, carbapenem inactivation method

Carbapenem-resistant *Enterobacteriaceae* (CRE), particularly those that are resistant due to carbapenemase production, are a significant threat to public health. Infections caused by CRE are associated with a high attributable mortality (1), and U.S. surveillance data have demonstrated a steady increase in the burden of disease caused by CRE since 2000 (2). The U.S. CRE epidemic is driven, in part, by *Klebsiella pneumoniae* isolates of sequence type (ST) 258 that express the *K. pneumoniae* carbapenemase (KPC) (3). Transfer of patients colonized or infected with this organism between health care institutions is thought to have led to the dissemination of ST 258 *K. pneumoniae* across the country. One particularly well documented outbreak described by the Centers for Disease Control and Prevention Epicenter Program demonstrated that patient transfers between 26 health care facilities across four counties lead to the spread of KPC-producing CRE to 40 patients over a 1-year period (4). Subsequent studies in this region documented that nearly 1 in 3 residents of long-term acute care facilities were colonized with KPC-producing CRE (5). CRE harboring other carbapenem-hydrolyzing enzymes, such as NDM, VIM, IMP, or the OXA-48 type, common in other areas of the world (6), are also spreading in the United States (7–9). The introduction of isolates expressing these

Received 25 January 2017 Returned for modification 13 February 2017 Accepted 21 March 2017

Accepted manuscript posted online 29 March 2017

Citation Miller SA, Hindler JA, Chengcuenca A, Humphries RM. 2017. Use of ancillary carbapenemase tests to improve specificity of phenotypic definitions for carbapenemase-producing *Enterobacteriaceae*. J Clin Microbiol 55:1827–1836. <https://doi.org/10.1128/JCM.00157-17>.

Editor Alexander J. McAdam, Boston Children's Hospital

Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to Romney M. Humphries, rhumphries@mednet.ucla.edu.

carbapenemases to a single institution by a patient traveling from an area of endemicity can lead to rapid spread, as occurred in our own institution when an OXA-232-producing *K. pneumoniae* strain was introduced to our facility by a colonized patient, leading to transmission of CRE to 14 patients via reprocessed duodenoscopes (10). A similar outbreak, caused by an NDM-producing *K. pneumoniae* strain in the Chicago area, was also recently documented as associated with the use of reprocessed duodenoscopes (11). In both outbreaks, rapid recognition of an unusual CRE type led to the epidemiological investigations that ultimately identified the source of the outbreak.

Early identification of patients colonized with carbapenemase-producing CRE (CP-CRE) and implementation of infection prevention strategies that include strict isolation practices have the potential to significantly slow the trajectory of CP-CRE dissemination in the United States (12–14). However, such interventions critically depend on accurate and rapid identification of patients colonized or infected by CRE through laboratory testing. Unfortunately, detection of CRE, let alone CP-CRE, remains a challenge for most clinical microbiology laboratories in the United States (6). For instance, updated carbapenem breakpoints for the *Enterobacteriaceae* published by the Clinical and Laboratory Standards Institute (CLSI) in 2010 have been incompletely adopted by clinical laboratories (15, 16), because not all manufacturers of antimicrobial susceptibility test (AST) systems have obtained U.S. Food and Drug Administration (FDA) clearance for these new breakpoints. And yet, the use of the 2010 breakpoints is imperative to accurately identify CRE (15). Bartsch and colleagues demonstrated through computer simulations that delayed implementation of updated carbapenem breakpoints may be the reason for a staggering 8,497 additional patients becoming colonized with CRE in a single region over the past 5 years (17).

In 2015, the CDC published a new surveillance definition for CRE in an attempt to help laboratories and epidemiologists to identify CP-CRE (18). This definition includes all members of the *Enterobacteriaceae* that are resistant to any carbapenem tested (ertapenem at ≥ 2 $\mu\text{g/ml}$, meropenem at ≥ 4 $\mu\text{g/ml}$, imipenem at ≥ 4 $\mu\text{g/ml}$, and/or doripenem at ≥ 4 $\mu\text{g/ml}$) and any isolate that harbors a carbapenemase gene or has a positive phenotypic test for carbapenemase. Some have concerns that this definition may overestimate CP-CRE because isolates resistant to ertapenem but not imipenem, meropenem, or doripenem often result from the presence of extended-spectrum beta-lactamases (ESBLs) or AmpC enzymes, which have some hydrolyzing activity against ertapenem, usually in combination with permeability defects. Similarly, some isolates without carbapenemases are resistant to imipenem, meropenem, and/or doripenem due to changes in porin proteins, often in conjunction with low-level AmpC or ESBL activities, albeit such isolates are less common (19). For instance, one study using the CDC definition found that 55% of isolates labeled as CP-CRE did not harbor carbapenemase genes (18). The authors suggested that the use of a second phenotypic test for carbapenemase, such as the modified Hodge test (MHT), improved specificity for the identification of CP-CRE. However, this was most effective for isolates of *K. pneumoniae* and *Escherichia coli*, whereas 26% of *Enterobacter* species isolates demonstrated false-positive MHT results (18). The MHT is also associated with false-negative results for NDM-producing CRE (6). While at the time, the MHT was the only CLSI-endorsed carbapenemase test that was readily performed by clinical laboratories, the CLSI voted in January 2017 to retire the MHT as a confirmatory test for CP-CRE and will remove this procedure in the 28th edition of the M100S standard, leaving laboratories with few practical options for testing CP-CRE.

Recently, two new carbapenemase detection methods have become available to clinical laboratories; one is a commercial assay for carbapenem resistance genes, and the other is a phenotypic test for carbapenemase activity that utilizes simple materials readily available in most clinical laboratories. A third method, the Carba-NP assay, has also been described, but this method requires the preparation of several reagents in-house and is not practical for clinical laboratories. The FDA-cleared Xpert Carba-R (Cepheid, Sunnydale, CA) is a multiplex PCR that detects the genes encoding the 5 most common carbapenemases, KPC, NDM, VIM, IMP, and OXA-48-like enzymes, directly from

TABLE 1 Categorical interpretations from broth microdilution MIC results for 125 isolates included in this study

Gene	Species	No. of isolates	% resistant/intermediate/% susceptible to ^a :		
			Ertapenem	Imipenem	Meropenem
<i>bla</i> _{IMP}	<i>E. cloacae</i>	1	100/0/0	0/1/0	0/1/0
	<i>K. pneumoniae</i>	1	100/0/0	100/0/0	100/0/0
<i>bla</i> _{KPC}	<i>Citrobacter freundii</i>	11	100/0/0	90.9/9.1/0	72.8/37.2/0
	<i>Citrobacter</i> spp.	1	100/0/0	100/0/0	100/0/0
	<i>E. cloacae</i>	2	100/0/0	100/0/0	100/0/0
	<i>E. coli</i>	5	100/0/0	75/25/0	50/25/25
	<i>Klebsiella oxytoca</i>	4	100/0/0	100/0/0	50/25/25
	<i>K. pneumoniae</i>	53	98/2/0	96.3/3.7/0	92.6/5.5/1.9
	<i>Raoultella planticola</i>	1	0/100/0	0/100/0	0/0/100
<i>bla</i> _{NDM}	<i>E. coli</i>	1	100/0/0	100/0/0	100/0/0
	<i>Providencia rettgeri</i>	1	100/0/0	100/0/0	100/0/0
<i>bla</i> _{OXA-48} like	<i>K. pneumoniae</i>	9	100/0/0	100/0/0	100/0/0
	<i>E. coli</i>	2	100/0/0	100/0/0	50/0/50
None	<i>C. freundii</i>	1	100/0/0	0/100/0	0/100/0
	<i>E. aerogenes</i>	8	75/0/25	50/50/0	12.5/37.5/50
	<i>E. cloacae</i>	7	85.7/14.3/0	28.6/14.3/57.1	14.3/14.3/71.4
	<i>E. coli</i>	7	71.4/28.6/0	14.3/28.6/57.1	42.9/14.3/42.8
	<i>K. pneumoniae</i>	9	88.9/0/11.1	44.5/22.2/33.3	55.6/22.2/22.2
	<i>S. marcescens</i>	1	0/0/100	100/0/0	0/0/100

^aThe CLSI breakpoints (M100S standard, 26th edition) (μg/ml) for susceptible, intermediate, and resistant interpretations are as follows: ertapenem, ≤0.5, 1, and ≥2; imipenem, ≤1, 2, and ≥4; and meropenem, ≤1, 2, and ≥4 (21).

bacterial colonies. This test is also FDA cleared for screening for patient colonization by rectal swab testing. The carbapenem inactivation method (CIM) was originally described by van der Zwaluw et al. to detect carbapenemase activity in Gram-negative bacilli using a cost-effective and sensitive approach (20). Additionally, a modified carbapenem inactivation method (mCIM) was recently evaluated by the CLSI and is described in detail in the new CLSI M100-S standard, 27th edition (CLSI, Wayne, PA) (21). At the time our study was performed, the mCIM method had not been evaluated by CLSI, and as such, the present study evaluates the performance of the Xpert Carba-R colony test, as well as the original CIM, with slight modifications to enable rapid performance of the test by clinical laboratories. The overall purpose was to evaluate the sensitivity and specificity of using both CDC's and UCLA's definition to identify CP-CRE with and without the use of CIM and Xpert ancillary assays for the detection of carbapenemase production in a laboratory that encounters a diverse population of CP-CRE.

RESULTS

A summary of the 125 isolates evaluated in this study is provided in Table 1. There were a total of 92 isolates positive for the presence of a carbapenemase gene, including 2 positive for *bla*_{IMP}, 77 for *bla*_{KPC}, 2 for *bla*_{NDM}, and 11 for *bla*_{OXA-48}-like genes, and 33 isolates had no carbapenem resistance genes detected. Among the *bla*_{OXA-48}-like containing isolates, 1 was subtyped as *bla*_{OXA-162}, 1 as *bla*_{OXA-181}, 1 as *bla*_{OXA-232}, and 8 as *bla*_{OXA-48}.

The CDC CRE surveillance definition was 98.9% sensitive (95% confidence interval [CI], 93.2 to 99.9%) and 6.1% specific (95% CI, 1.1 to 21.6%) for the presence of carbapenem resistance genes among this select collection of isolates compared to the results from in-house PCRs. In contrast, the UCLA definition was 100% sensitive (95% CI, 95.0 to 100%) and 24.2% specific (95% CI, 11.7 to 42.6%) (Table 2). One *K. pneumoniae* isolate that tested positive for *bla*_{KPC} did not meet the CDC surveillance definition criteria. This isolate had MICs of 1 μg/ml for ertapenem (susceptible), 2 μg/ml for

TABLE 2 Performance of CDC surveillance and UCLA CRE definitions for identification of CP-CRE with and without ancillary carbapenemase testing using Xpert Carba-R or CIM assay^a

Definition and ancillary test used	Sensitivity		Specificity	
	%	95% CI	%	95% CI
CDC CP-CRE				
No ancillary testing	98.9	93.2–99.9	6.1	1.1–21.6
With Xpert Carba-R	99.9	94.9–99.9	100	19.8–100
With CIM ^b	97.6	92.5–99.4	100	19.8–100
UCLA CRE				
No ancillary testing	100	95.0–100	24.2	11.7–42.6
With Xpert Carba-R	100	95.0–100	100	87.0–100
With CIM	97.7	91.4–99.6	100	87.0–100

^aThe CDC definition is ertapenem, meropenem, and/or imipenem resistant. The UCLA definition is intermediate or resistant to imipenem and/or meropenem, with the exception of *Proteus/Providencia/Morganella*, where only meropenem is considered. *n* = 125 isolates of *Enterobacteriaceae*; analysis with CIM ancillary testing excludes the 3 indeterminate results.

^bThe CIM ancillary test method consisted of incubating a meropenem disk in a TSB suspension of organisms for 2 h, followed by 18 h of incubation of disk diffusion plate.

imipenem (intermediate), and 1 $\mu\text{g/ml}$ for meropenem (susceptible). The presence of *bla*_{KPC} was confirmed by repeat testing by in-house PCRs, and the MICs were confirmed by repeat broth microdilution (BMD) testing. This isolate was positive by both the Xpert Carba-R for *bla*_{KPC} and the CIM test for the presence of a carbapenemase, which were also performed in duplicate.

Both the CDC and UCLA CRE definitions resulted in several false-positive results, 28 by the CDC definition and 25 by the UCLA definition. The CDC definition alone classified 5 isolates as CRE that were resistant to ertapenem but susceptible to imipenem and meropenem: one *E. coli*, three *Enterobacter cloacae*, and one *K. pneumoniae*. In contrast, the UCLA definition alone classified 2 *Enterobacter aerogenes* isolates as CRE that had intermediate imipenem MICs of 2 $\mu\text{g/ml}$ but were susceptible to meropenem and ertapenem. There were 23 isolates that were categorized as CRE using both the CDC and UCLA definitions that were negative for carbapenem resistance genes by PCR and carbapenemase activity by CIM (not shown).

Performance of the Xpert Carba-R assay. One isolate (negative for carbapenem resistance genes) gave an invalid result (0.8% of all isolates tested) on initial testing but yielded the correct negative result on repeat testing. After resolution, the Xpert Carba-R test was 100% sensitive and 100% specific for the detection of targeted carbapenem resistance genes in this select collection of isolates compared to the results of in-house PCR.

CIM test performed with incubation of organism and disks in TSB. On initial testing, 2 false-negative and 1 weakly positive (18 mm) results were observed with tryptic soy broth (TSB) after 6 h of incubation. The 2 false negatives (both *bla*_{OXA-48} positive) and 1 weak positive (*bla*_{KPC} positive) were all indeterminate at the 18- to 20-h reads, with microcolonies within the zones (Fig. 1). All 3 isolates were resistant to meropenem by BMD (Table 3). Following resolution, the CIM assay with TSB suspensions of organisms and 6 h of incubation was 94.6% sensitive (95% CI, 87.2 to 98.0%) and 100% specific (95% CI, 87 to 100%) (Table 4). At 18 to 20 h of incubation, the sensitivity was 97.7% (95% CI, 91.4 to 99.6%) and the specificity was 100% (95% CI, 87 to 100%), with a 2.4% rate of indeterminate results (Table 4). For 87 (94.6%) carbapenemase-positive isolates, there was 100% concordance between the results of both 6-h and 18-h incubations when no zone was recorded at 6 h of incubation.

CIM test performed with incubation of organism and disks in ddH₂O. During the initial testing, 9 false-negative results were observed at 6 h of incubation with the double-distilled water (ddH₂O) suspensions of organisms and meropenem disks. Five of the 9 isolates with false-negative results were falsely negative after repeat testing and indeterminate at 18 to 20 h. All 5 of these false negatives were *bla*_{OXA-48}-positive

FIG 1 Indeterminate CIM results using TSB (left) and ddH₂O (right) after 18 to 20 h of incubation for isolates 16-12-052 (A), 16-12-076 (B), and 16-12-098 (C).

isolates. Two of these false negatives were also falsely negative in TSB at 6 h (data not shown); the indeterminate 18- to 20-h results are shown in Fig. 1A and B. Another isolate (isolate 16-12-098) was positive in ddH₂O but negative in TSB at 6 h and was indeterminate in both ddH₂O and TSB at 18 to 20 h (Fig. 1C). The 4 remaining isolates with initial false-negative results were negative upon repeat testing (Table 3). All 4 isolates were resistant to meropenem by BMD (Table 4). Following resolution testing, CIM performed with ddH₂O yielded 88.0% sensitivity (95% CI, 79.2 to 93.6%) and 100% specificity (95% CI, 87.0 to 100%) at 6 h of incubation (Table 4). False-negative errors after resolution testing are summarized in Table 3 and included 1 isolate positive for

TABLE 3 Summary of discordant CIM results remaining after repeat testing

Isolate	Identification	Carbapenemase	MIC (μ g/ml) of ^a :			Result for CIM performed by incubating meropenem disk for 2 h in:			
						TSB suspension of organisms, followed by:		ddH ₂ O suspension of organisms, followed by:	
			ERT	IMI	MER	6-h plate incubation	18- to 20-h plate incubation	6-h plate incubation	18- to 20-h plate incubation
16-12-052	<i>E. coli</i>	OXA-48	>2	>8	>16	Neg	Indeterminate	Neg	Indeterminate
16-12-076	<i>K. pneumoniae</i>	OXA-48	>2	4	16	Neg	Indeterminate	Neg	Indeterminate
16-12-098	<i>K. pneumoniae</i>	KPC	>2	>8	>16	Neg	Indeterminate	Neg	Indeterminate
16-12-004	<i>P. rettgeri</i>	NDM	>2	>8	>16	Pos	Pos	Neg	Neg
16-12-055	<i>K. pneumoniae</i>	OXA-181	>2	4	4	Pos	Pos	Neg	Neg
16-12-057	<i>K. pneumoniae</i>	OXA-48	>2	>8	>16	Pos	Pos	Neg	Neg
16-12-081	<i>K. pneumoniae</i>	OXA-48	>2	4	16	Pos	Pos	Neg	Neg

^aIn-house broth microdilution MIC testing. ERT, ertapenem; IMI, imipenem; MER, meropenem; TSB, Trypticase soy broth.

TABLE 4 Summary of performance of CIM in comparison to reference carbapenemase PCR for identifying CP-CRE

CIM variable	% Sensitivity (95% CI)	% Specificity (95% CI)
Meropenem disk incubated in TSB, 6-h plate incubation	94.6 (87.2–98.0)	100 (87.0–100)
Meropenem disk incubated in TSB, 18- to 20-h plate incubation	97.7 (91.4–99.6)	100 (87.0–100)
Meropenem disk incubated in ddH ₂ O, 6-h plate incubation	88.0 (79.2–93.6)	100 (87.0–100)
Meropenem disk incubated in ddH ₂ O, 18- to 20-h plate incubation	93.0 (84.9–97.1)	100 (87.0–100)

*bla*_{NDM} and 3 positive for *bla*_{OXA-48}-like genes. After 18 to 20 h of incubation, all 9 isolates interpreted as false negative at 6 h of incubation were interpreted as negative, but upon repeat testing, 5 resolved as indeterminate, overall yielding 4 false negatives at both 6 h and 18 to 20 h of incubation. After resolution testing, CIM in ddH₂O had 93.0% sensitivity (95% CI, 84.9 to 97.1%) and 100% specificity (95% CI, 87.0 to 100%) for the 18- to 20-h readings, with a 4.8% rate of indeterminate results.

Use of ancillary testing to improve specificity of CDC CP-CRE definition. We evaluated the use of ancillary tests, i.e., the Xpert Carba-R and the CIM, to improve the specificity of the CDC and UCLA definitions for CPE. Xpert Carba-R, performed on isolates that screened positive for CPE by either the CDC or UCLA definitions, improved the specificity to 100%, with no loss in sensitivity (Table 2). Similarly, the use of the CIM improved the specificity to 100% for both definitions but with modest reductions in sensitivity to 97.6% for the CDC definition and 97.7% for the UCLA definition. In these calculations, indeterminate results were not considered for the CIM.

DISCUSSION

All clinical laboratories struggle with how to best detect CRE for clinical and epidemiological purposes. The revised CDC surveillance definition was associated with a sensitivity of 97.9% for the 97 carbapenemase producers evaluated by CDC when deriving the definition, very similar to the 98.9% sensitivity observed in the present single-center study of 92 carbapenemase-producing *Enterobacteriaceae*. The specificities for carbapenemase were only 21% in the initial studies by CDC and 6.1% in the present study. Of concern, the use of this highly sensitive but nonspecific definition in routine practice has the potential to negatively impact patient care, as many hospitals place patients who meet CRE definitions in contact precautions for life (22). Some states, including Illinois, have gone to added measures of tracking these patients via CRE registries to help expedite the implementation of contact precautions and enhanced infection control measures when these patients are admitted to a new health care facility. In addition, the CDC CRE Toolkit recommends that point prevalence studies be conducted when a new patient is identified with CRE. Preliminary data demonstrate that these drastic measures are promising to slow the spread of CRE but will only be effective if CRE are accurately identified.

In our hands, both the Xpert Carba-R and CIM improved the specificity of the CDC definition for CP-CRE to 100% (Table 2). The addition of these methods to the CDC definition may improve accurate identification of CP-CRE, without the potential collateral damage to patient care and resources associated with performing point prevalence studies for isolates of *Enterobacter* species that are resistant to ertapenem only due to resistance mechanisms other than carbapenemase production. The use of our alternative UCLA CRE definition detected 1 isolate missed by the CDC CRE surveillance definition, and others have similarly suggested the inclusion of imipenem- and meropenem-intermediate MICs in CRE definitions (23). However, the use of our alternative CRE definition did not greatly improve specificity, although it should be noted that only 8 of our institution's 60 ertapenem-resistant but meropenem- and imipenem-susceptible isolates were evaluated.

The CLSI recently published a procedure for mCIM in the 27th edition of the M100-S standard (21). The method evaluated herein differs from that endorsed by CLSI in several ways. The CLSI method uses 2 ml of TSB (versus 500 μ l) and incubates the meropenem disk-organism suspension for 4 h (versus 2 h) and the MHA plates for 18

to 24 h (versus 6 h, evaluated herein). The main reason we initiated these modifications was to enable performance of the CIM on a single shift in the laboratory, i.e., a minimum of 8 h of incubation versus 22 h of incubation time. However, it should be cautioned that we evaluated the test using a limited collection of CP-CRE and in a single laboratory. As such, prior to using these modifications, each laboratory should verify performance in their hands. Nonetheless, we feel confident that positive results, particularly when no zone is observed, can be trusted, whereas negatives may require the extended incubation. Of note, the authors of the original CIM method utilized ddH₂O to prepare organism suspensions. We noted several false-negative results when using this method (Table 3), and we recommend that laboratories use TSB, as endorsed by CLSI for the mCIM. The CIM may perform better in broth, as the isolates continue to grow and produce carbapenemase activity, although this theory was not evaluated in our study. Of concern are results from one isolate that harbored *bla*_{KPC} (isolate 16-12-98) and two isolates with *bla*_{OXA-48} (isolates 16-12-52 and 16-12-76) that yielded high carbapenem MICs *in vitro* but yielded zone sizes that would be considered negative for the CIM using TSB (Fig. 1A to C). This finding reinforces the need for an indeterminate interpretive category, as these three isolates all had colonies within the zone of inhibition.

There are advantages and limitations to both of the ancillary carbapenemase tests evaluated in our study. For instance, the Xpert Carba-R is more rapid than the CIM (<1 h versus 8 h), and requires very little hands-on technologist time. Informal polling of the technologists performing testing for this study revealed a clear preference for the Xpert Carba-R test over the CIM. Additionally, the Xpert Carba-R test allows laboratories and hospital epidemiologists to track the specific carbapenemases encountered in their facility. This may be of particular interest for institutions encountering international patients or domestic patients who sought care internationally, perhaps linked to medical tourism. This information is incredibly useful to identify potential outbreaks, in particular for those hospitals that encounter a high incidence of KPC-producing CRE, such as those in New York City and Los Angeles. In our particular experience with a duodenoscope-associated outbreak, the source was not identified until CRE-infected patients were stratified by the carbapenem resistance gene carried by the infecting organism (10).

The limitation to the Xpert Carba-R test (other than cost) is that the method is limited to the genes targeted by primers and probes. For instance, the Xpert Carba-R does not query for *bla*_{SME}, a carbapenemase gene found sporadically on the chromosome of isolates of *Serratia marcescens*, including in our own patient isolates (19). While considered less of an epidemiological concern because the gene is not harbored on a plasmid, outbreaks of isolates that harbor this gene have been documented (24, 25). While these isolates are typically susceptible to cephalosporins, derepression of the *S. marcescens* AmpC gene can result in pan-drug resistance, as we recently observed (P. Hemarajata, S. Yang, C. Holzman, K. Bush, J. A. Hindler, and R. M. Humphries, submitted for publication), leading to similar treatment dilemmas as are encountered for other CRE. Similarly, it is possible to imagine the emergence of a novel carbapenem resistance gene that is not detected by the Xpert Carba-R. This is more than theoretical, as both our in-house PCR and the first iteration of the Xpert Carba-R were unable to detect the OXA-181 and OXA-232 variants of the OXA-48 genes (26, 27). In contrast, the CIM method should detect any carbapenemase that hydrolyzes meropenem. However, the CIM is time consuming, although the use of our 6-h-incubation modification could potentially allow reporting of results within one shift by the laboratory, dependent on workflow.

It is important to comment that while the focus has been on CP-CRE, isolates that are carbapenem resistant by other mechanisms, such as porin mutation and plasmid AmpC, may also be of epidemiological importance. For example, a duodenoscope-associated CRE outbreak in Washington State was caused by an *E. coli* strain that harbored a plasmid-borne AmpC gene (28). These isolates can also be resistant to all antimicrobial classes and cause the same treatment dilemmas as CP-CRE (29). As such,

laboratories should not discount isolates that are very resistant (i.e., isolates with meropenem or imipenem MICs of $>8 \mu\text{g/ml}$) that test negative for carbapenemase genes. These should be submitted to public health laboratories for further analysis, either locally or at CDC.

Limitations to this study are that it was carried out in a single center, its design is nonprospective, and a relatively modest ($n = 125$) collection of isolates was included for evaluation. Another key limitation is the fact that isolates were chosen predominantly based on the UCLA CRE definition, with only 8 ertapenem-resistant, meropenem-/imipenem-susceptible isolates included. This undoubtedly resulted in a bias toward the UCLA definition for CRE. Nonetheless, both the UCLA and CDC definitions had very poor specificity for CP-CRE, and the key finding of this study was improved specificity of either definition with ancillary testing, a finding we feel was only minimally compromised by this selection bias. Future studies should evaluate more ertapenem-resistant, meropenem-/imipenem-susceptible isolates to fully appreciate the value of this category in the CP-CRE definition. Nonetheless, the results of this study were used to justify routine reflex testing of all isolates that meet the UCLA CRE definition for testing by the Xpert Carba-R assay. These results are routinely reported to infection control to aid with epidemiological tracking and investigations. The Xpert Carba-R test was chosen as opposed to the CIM due to both more rapid results and ease of use for laboratory staff.

MATERIALS AND METHODS

Bacterial isolates. A total of 125 isolates of *Enterobacteriaceae* isolated from 125 patients was evaluated. Isolates were selected using the UCLA CRE definition, i.e., not susceptible (intermediate or resistant, MIC of $\geq 2 \mu\text{g/ml}$) to meropenem and/or imipenem, as defined by the current CLSI M100-S standard (27th edition) (21). Isolates of *Proteus*, *Providencia*, and *Morganella* that were not susceptible to imipenem alone were excluded. Of the 125 isolates, 8 were prospective isolates that met the CDC CRE definition but not the UCLA definition (i.e., resistant to ertapenem but susceptible to meropenem and imipenem). Susceptibility testing was performed according to the CLSI reference broth microdilution (BMD) method on panels prepared in-house. The isolates were recovered from urine ($n = 40$), blood ($n = 23$), respiratory secretion ($n = 29$), wound/tissue ($n = 9$), and aspirate/fluid ($n = 16$) samples from UCLA patients between 2014 and 2016. Eight isolates (all positive for *bla*_{OXA-48}-like genes) were provided by The Medicines Company (Parsippany, NJ). All isolates were stored at -70°C in brucella broth with 15% glycerol (Hardy Diagnostics, Valencia, CA) and were subcultured twice on blood agar plates (BBL; BD, Sparks MD) prior to testing.

CIM. The CIM method was performed according to the methods described by van der Zwaluw and colleagues (20), with some modifications. Briefly, 500 μl of sterile double-distilled water or tryptic soy broth (TSB; BD, Sparks MD) was added to a 1.5-ml sterile microcentrifuge tube. For each CRE isolate, a heaping 1- μl loopful of organisms from an 18- to 24-h culture on a blood agar plate was added to the broth or water and vortexed to obtain a homogenous suspension. To this, a 10- μg meropenem disk (BD) was added, ensuring the disk was immersed in the suspension. The suspension was then incubated at 35°C for 2 h in an ambient air incubator. After incubation, the disk was carefully removed from the suspension. It was then applied to a lawn of *Escherichia coli* ATCC 25922 on a Mueller-Hinton agar (MHA) plate (BD) that had been inoculated as for a routine disk diffusion test, using a suspension equivalent to 0.5 McFarland. The plates were incubated at 35°C in ambient air for 6 h, after which time the zones of growth inhibition surrounding the meropenem disk were measured using reflected light. Within 15 min, the plates were reincubated for an additional 12 to 14 h (total 18 to 20 h of incubation), and the zone sizes were measured again. A zone of ≤ 15 mm was considered positive for carbapenemase production, and a clear zone of ≥ 19 mm was considered negative. Zones of 16 to 18 mm or ≥ 19 mm with colonies present within the zone were considered indeterminate (Fig. 1).

Quality control was performed by testing a carbapenemase-positive (*K. pneumoniae* ATCC BAA-1705) and a carbapenemase-negative (*K. pneumoniae* ATCC BAA-1706) control strain on each day of testing. For additional controls, meropenem disks were incubated in TSB or water alone (no organisms) for 2 h at 35°C . These and a dry meropenem disk were also applied to the *E. coli* lawn, and zones were evaluated at 18 to 20 h of incubation to ensure they fell within CLSI quality control (QC) ranges for meropenem and *E. coli* ATCC 25922 (21).

Detection of carbapenem resistance genes. All isolates were tested using in-house-developed TaqMan PCRs for the detection of *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{SME}, and *bla*_{OXA-48} (19, 26). Isolates positive for *bla*_{OXA-48} were subtyped using a high-resolution melt assay, as described previously (26). Quality control included testing an isolate positive or negative for each target on each day of testing. Additionally, a 16S rRNA gene internal control was included in each PCR. Isolates were tested by the Xpert Carba-R assay (Cepheid) according to the manufacturer's instructions. Quality control was performed according to the Xpert package insert.

Study design and data analysis. CIM and Xpert Carba-R tests were performed on the same day using the same suspension of organisms. Sensitivity and specificity were calculated for the CDC CP-CRE

definition and for the UCLA CRE definition, using results previously obtained from an in-house-developed carbapenem resistance gene PCR as the gold standard. Results for isolates classified as CPE by MIC interpretations were considered false positive if the isolate was negative for carbapenem resistance genes by the in-house PCR assay. Similarly, results were false negative if the isolate produced a carbapenemase or carried a carbapenem resistance gene but did not fit the CRE definition based on interpretive MIC results. Sensitivity and specificity were recalculated following the inclusion of results from the CIM and Xpert assays. Testing of isolates with discrepant results was repeated, and if the errors were corrected, they were not included in the final data analysis.

ACKNOWLEDGMENTS

Xpert Carba-R kits for this study were provided by Cepheid. We thank Olga Lovmovska at the Medicines Company for providing some isolates for this study.

REFERENCES

- Kaye KS, Pogue JM. 2015. Infections caused by resistant gram-negative bacteria: epidemiology and management. *Pharmacotherapy* 35: 949–962. <https://doi.org/10.1002/phar.1636>.
- Centers for Disease Control and Prevention. 2013. Vital signs: carbapenem-resistant Enterobacteriaceae. *MMWR Morb Mortal Wkly Rep* 62:165–170.
- Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y, Brolund A, Giske CG. 2009. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob Agents Chemother* 53: 3365–3370. <https://doi.org/10.1128/AAC.00126-09>.
- Won SY, Munoz-Price LS, Lolans K, Hota B, Weinstein RA, Hayden MK, Centers for Disease Control and Prevention Epicenter Program. 2011. Emergence and rapid regional spread of *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae. *Clin Infect Dis* 53: 532–540. <https://doi.org/10.1093/cid/cir482>.
- Lin MY, Lyles-Banks RD, Lolans K, Hines DW, Spear JB, Petrak R, Trick WE, Weinstein RA, Hayden MK, Centers for Disease Control and Prevention Epicenter Program. 2013. The importance of long-term acute care hospitals in the regional epidemiology of *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae. *Clin Infect Dis* 57: 1246–1252. <https://doi.org/10.1093/cid/cit500>.
- Miller S, Humphries RM. 2016. Clinical laboratory detection of carbapenem-resistant and carbapenemase-producing Enterobacteriaceae. *Expert Rev Anti Infect Ther* 14:705–717. <https://doi.org/10.1080/14787210.2016.1206815>.
- Lyman M, Walters M, Lonsway D, Rasheed K, Limbago B, Kallen A. 2015. Notes from the field: carbapenem-resistant Enterobacteriaceae producing OXA-48-like carbapenemases—United States, 2010–2015. *MMWR Morb Mortal Wkly Rep* 64:1315–1316. <https://doi.org/10.15585/mmwr.mm6447a3>.
- Centers for Disease Control and Prevention. 2010. Update: detection of a verona integron-encoded metallo-beta-lactamase in *Klebsiella pneumoniae*—United States, 2010. *MMWR Morb Mortal Wkly Rep* 59:1212.
- Centers for Disease Control and Prevention. 2017. Tracking CRE. CDC, Atlanta, GA. <https://www.cdc.gov/hai/organisms/cre/trackingcre.html>. Accessed 23 January 2017.
- Yang S, Hemarajata P, Hindler JA, Li F, Adisetiyo H, Aldrovandi G, Sebra R, Kasarskis A, MacCannell D, Didelot X, Russell D, Rubin Z, Humphries R. 2017. Evolution and transmission of carbapenem-resistant *Klebsiella pneumoniae* expressing the bla_{OXA-232} gene during an institutional outbreak associated with endoscopic retrograde cholangiopancreatography. *Clin Infect Dis* 64:894–901. <https://doi.org/10.1093/cid/ciw876>.
- Epstein L, Hunter JC, Arwady MA, Tsai V, Stein L, Gribogiannis M, Frias M, Guh AY, Laufer AS, Black S, Pacilli M, Moulton-Meissner H, Rasheed JK, Avillan JJ, Kitchel B, Limbago BM, MacCannell D, Lonsway D, Noble-Wang J, Conway J, Conover C, Vernon M, Kallen AJ. 2014. New Delhi metallo-beta-lactamase-producing carbapenem-resistant *Escherichia coli* associated with exposure to duodenoscopes. *JAMA* 312:1447–1455. <https://doi.org/10.1001/jama.2014.12720>.
- Hayden MK, Lin MY, Lolans K, Weiner S, Blom D, Moore NM, Fogg L, Henry D, Lyles R, Thurlow C, Sikka M, Hines D, Weinstein RA, Centers for Disease Control and Prevention Epicenter Program. 2015. Prevention of colonization and infection by *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae in long-term acute-care hospitals. *Clin Infect Dis* 60:1153–1161. <https://doi.org/10.1093/cid/ciu1173>.
- Enfield KB, Huq NN, Gosseling MF, Low DJ, Hazen KC, Toney DM, Slitt G, Zapata HJ, Cox HL, Lewis JD, Kundzins JR, Mathers AJ, Sifri CD. 2014. Control of simultaneous outbreaks of carbapenemase-producing Enterobacteriaceae and extensively drug-resistant *Acinetobacter baumannii* infection in an intensive care unit using interventions promoted in the Centers for Disease Control and Prevention 2012 Carbapenemase-Resistant Enterobacteriaceae Toolkit. *Infect Control Hosp Epidemiol* 35: 810–817. <https://doi.org/10.1086/676857>.
- Lee BY, Bartsch SM, Wong KF, McKinnell JA, Slayton RB, Miller LG, Cao C, Kim DS, Kallen AJ, Jernigan JA, Huang SS. 2016. The potential trajectory of carbapenem-resistant Enterobacteriaceae, an emerging threat to health-care facilities, and the impact of the Centers for Disease Control and Prevention Toolkit. *Am J Epidemiol* 183:471–479. <https://doi.org/10.1093/aje/kwv299>.
- Thaden JT, Lewis SS, Hazen KC, Huslage K, Fowler VG, Jr, Moehring RW, Chen LF, Jones CD, Moore ZS, Sexton DJ, Anderson DJ. 2014. Rising rates of carbapenem-resistant Enterobacteriaceae in community hospitals: a mixed-methods review of epidemiology and microbiology practices in a network of community hospitals in the southeastern United States. *Infect Control Hosp Epidemiol* 35:978–983. <https://doi.org/10.1086/677157>.
- McKinnell JA, Hindler J, Epton E, Horwich-Scholefield S, Miller LG, Mendez J, Martinez J, Sinkowitz J, Terashita D, Marquez P, Bhauria S, Moran M, Pandes L, Hershey C, Humphries R. 2016. Incomplete adoption of clinical laboratory standards institute (CLSI) breakpoints to detect carbapenem resistant organisms, poster 357. IDWeek 2016, New Orleans, LA, 26 to 30 October 2016.
- Bartsch SM, Huang SS, Wong KF, Slayton RB, McKinnell JA, Sahm DF, Kazmierczak K, Mueller LE, Jernigan JA, Lee BY. 2016. Impact of delays between Clinical and Laboratory Standards Institute and Food and Drug Administration revisions of interpretive criteria for carbapenem-resistant Enterobacteriaceae. *J Clin Microbiol* 54:2757–2762. <https://doi.org/10.1128/JCM.00635-16>.
- Chea N, Bulens SN, Kongphet-Tran T, Lynfield R, Shaw KM, Vagnone PS, Kainer MA, Muleta DB, Wilson L, Vaeth E, Dumyati G, Concannon C, Phipps EC, Culbreath K, Janelle SJ, Bamberg WM, Guh AY, Limbago B, Kallen AJ. 2015. Improved phenotype-based definition for identifying carbapenemase producers among carbapenem-resistant Enterobacteriaceae. *Emerg Infect Dis* 21:1611–1616. <https://doi.org/10.3201/eid2109.150198>.
- Pollett S, Miller S, Hindler J, Uslan D, Carvalho M, Humphries RM. 2014. Phenotypic and molecular characteristics of carbapenem-resistant Enterobacteriaceae in a health care system in Los Angeles, California, from 2011 to 2013. *J Clin Microbiol* 52:4003–4009. <https://doi.org/10.1128/JCM.01397-14>.
- van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. 2015. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. *PLoS One* 10:e0123690. <https://doi.org/10.1371/journal.pone.0123690>.
- CLSI. 2017. Performance standards for antimicrobial susceptibility testing, 27th ed. M100-S27. Clinical and Laboratory Standards Institute, Wayne, PA.
- Humphries RM, McKinnell JA. 2015. Continuing challenges for the clinical laboratory for detection of carbapenem-resistant Enterobacteriaceae. *J Clin Microbiol* 53:3712–3714. <https://doi.org/10.1128/JCM.02668-15>.
- Tamma PD, Huang Y, Opene BN, Simner PJ. 2016. Determining the

- optimal carbapenem MIC that distinguishes carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae. *Antimicrob Agents Chemother* 60:6425–6429. <https://doi.org/10.1128/AAC.00838-16>.
24. Bush K, Pannell M, Lock JL, Queenan AM, Jorgensen JH, Lee RM, Lewis JS, Jarrett D. 2013. Detection systems for carbapenemase gene identification should include the SME serine carbapenemase. *Int J Antimicrob Agents* 41:1–4. <https://doi.org/10.1016/j.ijantimicag.2012.08.008>.
25. Queenan AM, Torres-Viera C, Gold HS, Carmeli Y, Eliopoulos GM, Moelling RC, Jr, Quinn JP, Hindler J, Medeiros AA, Bush K. 2000. SME-type carbapenem-hydrolyzing class A beta-lactamases from geographically diverse *Serratia marcescens* strains. *Antimicrob Agents Chemother* 44:3035–3039. <https://doi.org/10.1128/AAC.44.11.3035-3039.2000>.
26. Hemarajata P, Yang S, Hindler JA, Humphries RM. 2015. Development of a novel real-time PCR assay with high-resolution melt analysis to detect and differentiate OXA-48-like beta-lactamases in carbapenem-resistant Enterobacteriaceae. *Antimicrob Agents Chemother* 59:5574–5580. <https://doi.org/10.1128/AAC.00425-15>.
27. Decousser JW, Poirel L, Desroches M, Jayol A, Denamur E, Nordmann P. 2015. Failure to detect carbapenem-resistant *Escherichia coli* producing OXA-48-like using the Xpert Carba-R assay. *Clin Microbiol Infect* 21:e9–e10. <https://doi.org/10.1016/j.cmi.2014.09.006>.
28. Wendorf KA, Kay M, Baliga C, Weissman SJ, Gluck M, Verma P, D'Angeli M, Swoveland J, Kang MG, Eckmann K, Ross AS, Duchin J. 2015. Endoscopic retrograde cholangiopancreatography-associated AmpC *Escherichia coli* outbreak. *Infect Control Hosp Epidemiol* 36:634–642. <https://doi.org/10.1017/ice.2015.66>.
29. Goodman KE, Simner PJ, Tamma PD, Milstone AM. 2016. Infection control implications of heterogeneous resistance mechanisms in carbapenem-resistant Enterobacteriaceae (CRE). *Expert Rev Anti Infect Ther* 14:95–108. <https://doi.org/10.1586/14787210.2016.1106940>.