Clinical Chemistry 63:3
000–000 (2016)

Infectious Disease

Evaluation of Genotypic and Phenotypic Methods to Detect Carbapenemase Production in Gram-Negative Bacilli

Allison R. McMullen,[1] Melanie L. Yarbrough,[1] Meghan A. Wallace,[1] Angela Shupe,[1] and Carey-Ann D. Burnham[1*]

BACKGROUND: Carbapenemase-producing gram-negative bacteria (CP-GNB) are an urgent and expanding public health threat. Rapid and accurate identification of these organisms facilitates infection prevention efforts in healthcare facilities. The objective of our study was to evaluate methods to detect and identify CP-GNB.

METHODS: We examined 189 carbapenem-resistant GNB (CR-GNB), including *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* complex, using 3 different methods: 2 methods to screen isolates of GNB for carbapenemase production [the carbapenem inactivation method (CIM) and 2 chromogenic agars] and a molecular method (Cepheid GeneXpert Carba-R) to identify the mechanism of carbapenem resistance and the associated resistance genes (bla_{KPC}, bla_{NDM}, bla_{IMP}, $bla_{OXA\text{-}48\text{-}like}$, and bla_{VIM}).

RESULTS: The CIM was a simple and inexpensive phenotypic screen to differentiate between CR-GNB and CP-GNB, with improved analytical performance characteristics and interreader correlation compared to the modified Hodge test. Both chromogenic agars evaluated (HardyCHROM CRE and chromID CARBA) were able to support growth of most of the organisms tested, including isolates possessing the $bla_{OXA\text{-}48\text{-}like}$ gene. However, these media had a low analytical specificity for carbapenemase production, with breakthrough of CR-GNB that did not produce carbapenemase. The Xpert Carba-R assay was rapid and easy to perform, and demonstrated 100% positive and negative agreement for characterization of genetic determinants of carbapenem resistance.

CONCLUSIONS: Screening by CIM followed by the Xpert Carba-R PCR is an accurate method for detecting and characterizing CP-GNB, including *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* complex.

The rapid emergence and expansion of carbapenem-resistant gram-negative bacteria (CR-GNB)[2] is an urgent global public health threat *(1, 2)*. Carbapenemases, or enzymes that can degrade broad-spectrum β-lactams including carbapenems, can be located on mobile genetic elements, facilitating the spread of resistance genes between organisms. Although numerous carbapenemase genes have been described to date, the most common determinants conferring carbapenem resistance in the US include bla_{KPC} (Ambler class A), bla_{NDM} (Ambler class B), and $bla_{OXA\text{-}48\text{-}like}$ (Ambler class D) *(3–5)*. These enzymes are most frequently isolated from *Enterobacteriaceae*, including *Klebsiella pneumoniae*, *Enterobacter* spp., and *Escherichia coli*. Additionally, they have been identified in other GNB, including *Pseudomonas aeruginosa* and *Acinetobacter* spp. *(3, 6, 7)*. Accurate detection and characterization of carbapenemase-producing GNB (CP-GNB) can inform infection-prevention measures, be important for epidemiological purposes, and help expedite appropriate therapy in infected patients. For example, ceftazidime-avibactam has activity against Ambler classes A and C β-lactamases, but not Ambler classes B and D *(8–10)*.

Methods in the clinical microbiology laboratory for the detection of CP-GNB rely on phenotypic detection of carbapenem resistance in concert with molecular studies to identify and characterize the genetic determinants conferring carbapenem resistance. Historically, the modified Hodge test (mHT) was recommended as a first-line method to detect carbapenemase production in *Enterobacteriaceae (11)*. However, this method has important

[1] Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO.
* Address correspondence to this author at: Department of Pathology & Immunology, Washington University School of Medicine, 660 S Euclid Ave., Campus Box 8118, St. Louis, MO 63110. Fax 314-454-2274; e-mail cburnham@path.wustl.edu.
Received August 1, 2016; accepted October 10, 2016.
Previously published online at DOI: 10.1373/clinchem.2016.264804

[2] Nonstandard abbreviations: CR-GNB, carbapenem-resistant gram-negative bacteria; CP-GNB, carbapenemase-producing GNB; mHT, modified Hodge test; ESBL, extended-spectrum β-lactamase; CIM, carbapenem inactivation method; McF, McFarland; TSB, tryptic soy broth; PPA, positive percent agreement; NPA, negative percent agreement.

Table 1. Overview of bacterial isolates evaluated.

Organism	Total	Carbapenemase gene							
		bla_{KPC}	bla_{NDM}	$bla_{OXA-48-like}$	bla_{VIM}	bla_{IMP}	bla_{SME}	bla_{IMI}	None
K. pneumoniae	83	53	8	11	2	0	0	0	9
E. cloacae complex	28	14	1	0	1	0	0	2	10
P. aeruginosa	19	0	0	0	4	0	0	0	15
E. coli	15	5	9	0	0	0	0	0	1
A. baumannii complex	11	0	4	0	0	0	0	0	7
E. aerogenes	7	0	0	1	0	1	0	0	5
K. oxytoca	6	5	0	0	0	0	0	0	1
S. marcescens	6	0	0	0	0	0	6	0	0
P. mirabilis	2	1	1	0	0	0	0	0	0
P. rettgeri	3	0	2	0	0	0	0	0	1
Citrobacter freundii	2	2	0	0	0	0	0	0	0
Morganella morganii	2	2	0	0	0	0	0	0	0
Citrobacter spp.	1	0	1	0	0	0	0	0	0
H. alvei	1	0	0	0	0	0	0	0	1
Kluyvera ascorbata	1	1	0	0	0	0	0	0	0
Raoultella ornithinolytica	1	1	0	0	0	0	0	0	0
Salmonella spp.	1	0	1	0	0	0	0	0	0
All	189	84	27	12	7	1	6	2	50

limitations, both with regard to analytical sensitivity and specificity. For example, false-negative results are commonly reported for metallo-β-lactamases, such as bla_{NDM}, and false-positive results are reported in isolates with compound antimicrobial resistance phenotypes, such as AmpC and/or extended-spectrum β-lactamase (ESBL) enzymes with a porin mutation *(12)*. In addition, a positive mHT does not inform the genetic determinant conferring carbapenem resistance. Our objective in this investigation was to evaluate 3 emerging methods to detect carbapenem resistance in GNB—the carbapenem inactivation method (CIM), Cepheid Xpert Carba-R, and chromogenic agar.

Materials and Methods

BACTERIAL ISOLATES

One hundred and twenty bacterial strains (98 *Enterobacteriaceae*, 15 *P. aeruginosa*, and 7 *Acinetobacter baumannii* complex) were obtained from archived clinical isolates at Barnes-Jewish Hospital (St. Louis, MO). These isolates were originally recovered between 2011 and 2016 from different specimen types, including urine, blood, respiratory specimens, body fluids, tissues, and wounds. All isolates previously tested as not susceptible (i.e., intermediate or resistant) to meropenem using Kirby–Bauer disk diffusion (disk diffusion zone of ≤22 mm) *(12)*. For the purpose of evaluation herein, all nonsusceptible isolates are described here as "resistant." Additional isolates from the *Enterobacteriaceae* Carbapenemase Diversity Panel were obtained from the CDC Antimicrobial Resistance Isolate Bank (Atlanta, GA) *(13)*. An additional 18 clinical isolates from our laboratory's strain bank were evaluated. An overview of all isolates and carbapenemase genes evaluated is found in Table 1. For all tests, the following organisms were used for quality control: *K. pneumoniae* ATCC BAA-1705 (bla_{KPC} positive strain), *K. pneumoniae* ATCC 700603 (ESBL-positive, carbapenemase-negative strain), and *E. coli* ATCC 29522 (carbapenem-susceptible strain).

BACTERIAL CULTIVATION

From frozen stock, each isolate was inoculated onto blood agar plates (Hardy Diagnostics). A 10-μg meropenem disk (Becton, Dickinson and Company) was placed in the first quadrant to confirm retention of meropenem resistance upon storage and subculture. Isolates were suspended in normal saline to a density of 0.5 McFarland (McF) standard *(14)* for workup; the same suspension was used for each of the assays evaluated.

CARBAPENEM INACTIVATION METHOD

Using a method modified from van der Zwaluw et al. *(15)*, a heaping 10-μL loop of each isolate was suspended

in 400 μL tryptic soy broth (TSB) in a 1.5 mL Eppendorf tube. Growth nearest to the meropenem disk on the subculture plate was selected for analysis. The suspension was vortex-mixed for 10 s and a 10 μg meropenem disk was added to each tube and vortex-mixed again for 10–30 s. Tubes were incubated at 35 °C in air for 3–4 h. After incubation, each disk was placed onto a Mueller–Hinton agar plate inoculated with a 0.5 McF standard of *E. coli* ATCC 29522 (a meropenem-susceptible strain). Up to 8 disks were placed onto each 150-mm plate. As a control, a meropenem disk incubated in TSB without any organism was also tested each time testing was performed. Plates were incubated at 35 °C in air and the zone around the meropenem disk was measured and recorded at 6 h and again after an overnight incubation (18–24 h). A positive result for a carbapenemase-producing isolate was indicated by uninhibited growth of the *E. coli* (evaluated using a zone size ≤8 mm). A negative result for carbapenemase production resulted when a large zone of inhibition of *E. coli* was observed around the meropenem disk (zone size ≥15 mm). Based on preliminary testing, a meropenem zone size of 9–14 mm was classified as indeterminate (data not shown). For isolates requiring additional testing to query for production of a metallo-β-lactamase, 50 μL of 0.5 mol/L EDTA was added to the TSB with a meropenem disk and the test organism and incubated as described above (CIM-EDTA). A positive result (suggestive of a metallo-β-lactamase) was a zone size difference of ≥5 mm around the meropenem disk for the isolate incubated with EDTA as compared to without EDTA. The instructions for this procedure are included in the Supplemental Methods file that accompanies the online version of this article at http://www.clinchem.org/content/vol63/issue3.

CIM TO mHT COMPARISON STUDY

To compare the performance characteristics of the CIM method to the mHT, a subset of 20 organisms representing a variety of resistance determinants (6 bla_{KPC}, 3 bla_{NDM}, 3 bla_{VIM}, 3 $bla_{OXA-48-like}$, 1 bla_{IMP}, and 4 carbapenemase-negative, meropenem-resistant isolates) were tested using both the CIM and mHT. The mHT was set up per the protocol in the CLSI M100-26S document *(12)*. Ten experienced microbiologists interpreted both assays.

EVALUATION OF GROWTH ON CHROMOGENIC AGAR

HardyCHROM CRE Agar (Hardy Diagnostics) and chromID CARBA Agar (bioMérieux) were evaluated. Using a Pasteur pipette, 2 drops of a 0.5 McF standard of each isolate were placed onto each plate and streaked using a 4-quadrant method for isolation. Each plate was incubated at 35 °C in air and protected from the light. Plates were examined at 24 and 48 h and relevant growth characteristics, including colony color and a semiquantitative assessment of growth (i.e., 1+ to 4+), were recorded for each strain.

CEPHEID Xpert Carba-R ASSAY

The Xpert Carba-R assay detects and differentiates 5 carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{IMP}, $bla_{OXA-48-like}$, and bla_{VIM}). According to manufacturer's directions, a 10-μL loopful of the 0.5 McF standard suspension of each organism was added to a 5 mL vial of Xpert Carba-R Sample Reagent and vortex-mixed for 10 s *(16)*. Using the provided transfer pipette, the sample reagent was added to the Xpert Carba-R cartridge and analyzed using the Cepheid GeneXpert platform (Cepheid).

PCR ASSAYS FOR bla_{KPC}, bla_{NDM}, bla_{IMP}, $bla_{OXA-48-like}$, AND bla_{VIM}

All isolates (except those provided by the CDC as part of the *Enterobacteriaceae* Carbapenemase Diversity Panel) were tested for the bla_{KPC}, bla_{NDM}, bla_{IMP}, and/or bla_{VIM} genes using individual laboratory-developed real-time PCR assays using the Cepheid SmartCycler II (Cepheid) as previously described *(17–20)*. The procedure for $bla_{OXA-48-like}$ detection was adapted from the CDC protocol and used the following primers: OXA-48-FWD180, ACG GGC GAA CCA AGC AT; OXA-48-REV239, GCG ATC AAG CTA TTG GGA ATT A; and OXA-PROBE199, FAM-TT ACC CGA ATC TAC C-BHQ.

STATISTICAL ANALYSES

For each assay evaluated, positive percent agreement (PPA) and negative percent agreement (NPA) were calculated using an aggregate gold standard as follows. For a positive result, the isolate must be meropenem intermediate or resistant and be positive for a carbapenemase gene using the laboratory-developed PCR assays or must have been previously characterized as part of the CDC Carbapenemase Diversity Panel as possessing a carbapenemase. For a negative result, meropenem susceptibility or negativity for a carbapenemase gene must be confirmed by the laboratory-developed PCR assays or per the CDC Carbapenemase Diversity Panel *(21)*.

Results

CARBAPENEM INACTIVATION METHOD

Of the 189 meropenem-resistant GNB evaluated, all isolates possessing a carbapenemase gene (bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{IMP}, bla_{VIM}, bla_{SME}, or bla_{IMI}) by PCR or by the CDC reference panel were positive by the CIM (zone around meropenem disk of ≤8 mm), with the exception of 1 isolate of *Proteus mirabilis* possessing the bla_{NDM} gene, which was indeterminate due to the swarming characteristics of this species. Zone sizes for the *Enterobacteriaceae* isolates could easily be interpreted af-

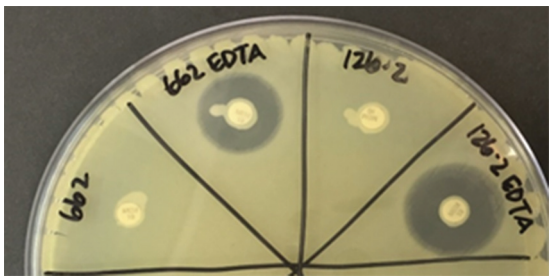

Fig. 1. CIM-EDTA for the detection of metallo-β-lactamases. *P. rettgeri* isolates with an uncharacterized carbapenemase (662) and bla_{NDM} determinant (126–2) incubated with and without EDTA.

Growth immediately around the meropenem disk represents the test organism with which the disk was incubated.

ter a 6-h incubation period and were sustained following overnight incubation. *P. aeruginosa* isolates that contained the bla_{VIM} gene (n = 4) were difficult to interpret after the 6-h incubation, with zones of 12–16 mm, but after the overnight incubation all zones measured 6–8 mm. One *Providencia rettgeri* isolate and 5 *A. baumannii* complex isolates, all of which tested negative by both the Xpert Carba-R assay and the real-time PCRs for bla_{KPC}, bla_{NDM}, and $bla_{OXA-48-like}$, were positive by the CIM. Isolates that were negative by PCR, but positive by the CIM, were evaluated using the CIM-EDTA, which tests for the presence of a metallo-β-lactamase (Fig. 1). The carbapenemase produced by the *P. rettgeri* isolate and 5 of 6 of the *A. baumannii* complex isolates were inactivated with the addition of EDTA. The addition of EDTA to the TSB with the meropenem disk inhibited carbapenemase activity, leading to a larger zone around the disk (18 mm with EDTA vs 6 mm without EDTA for the *P. rettgeri*; 15–18 mm with EDTA vs 6–7 mm without EDTA for the *A. baumannii* complex). Accurate performance of the CIM-EDTA was confirmed using bla_{NDM} and bla_{VIM} (metallo-β-lactamases) positive isolates. Overall, the CIM demonstrated 99.3% PPA and 86.0% NPA for carbapenemase production with the aggregate reference standard (Table 2).

A comparison of interpretations of the CIM vs the mHT is shown in Table 3. Although the mHT is described by CLSI for *Enterobacteriaceae* and not for nonfermenting GNB, isolates of *P. aeruginosa* (n = 3) and *Acinetobacter* spp. (n = 1) were included in this analysis for consistency in testing and to capture all of the resistance mechanisms included in this study. For the mHT, *P. aeruginosa* isolates possessing a bla_{VIM} (n = 3) were difficult to interpret due to the presence of a zone of lighter "haze" of growth up to the meropenem disk. A correct interpretation was provided for all isolates with a bla_{KPC}, $bla_{OXA-48-like}$, and bla_{IMP} gene. In contrast, 8 of 10 readers interpreted the bla_{NDM}-positive isolates as negative, while the other readers interpreted the isolate as positive (n = 1) and indeterminate (n = 1). For CR-GNBs that were negative for a carbapenemase, all 4 isolates were interpreted as negative by the CIM, while some readers interpreted these isolates as positive or indeterminate using the mHT (Table 3). Overall, there was agreement between technologists for 16/20 isolates for the CIM and 11/20 isolates for the mHT. For the CIM with this subset of isolates, the PPA and NPA were 97.5% and 100%, respectively, while for the mHT the PPA was 75% and the NPA was 58.5%. For the purpose of these calculations, indeterminate results were considered positive, since carbapenemase production would not be ruled out and these isolates would require additional characterization/workup. The CIM facilitated ease of correct interpretations as expected for all *Enterobacteriaceae*.

EVALUATION OF THE Xpert Carba-R ASSAY

We evaluated the analytical performance characteristics of the Xpert Carba-R assay for the detection of bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{IMP}, and bla_{VIM} genes. The Xpert Carba-R assay has a hands-on time of approximately 1–2 min per sample with an analysis time of less than 1 h *(16)*. Positive results were obtained for all isolates previously characterized as possessing the carbapen-

Table 2. Performance characteristics of methods evaluated for the detection of CP-GNB.

Method	Overall percent agreement, %	PPA, %	NPA, %
CIM	95.7	99.3	86.0
HardyCHROM CRE	73.0	99.3	0.0
bioMérieux chromID	79.9	99.3	25.0
Xpert Carba-R	95.8[a]/100.0[b]	94.2[a]/100.0[b]	100.0[a]/100.0[b]

[a] Calculation based on all carbapenemase genes, including those not detected by the Xpert Carba-R assay (e.g., bla_{SME}, bla_{IMI}).
[b] Calculations based only on carbapenemase genes detectable by the Xpert Carba-R assay (bla_{KPC}, bla_{NDM}, bla_{IMP}, $bla_{OXA-48-like}$, and bla_{VIM}).

Table 3. CIM vs mHT.[a]

		Interpretation, n					
		Positive		Negative		Indeterminate[b]	
Organism	Genetic determinant	CIM	mHT	CIM	mHT	CIM	mHT
K. pneumoniae	bla_{KPC}	10	10				
K. pneumoniae	bla_{KPC}	10	10				
K. pneumoniae	bla_{KPC}	10	10				
K. pneumoniae	bla_{KPC}	10	10				
E. cloacae complex	bla_{KPC}	10	10				
E. cloacae complex	bla_{KPC}	10	10				
E. coli	bla_{NDM}	10	1		8		1
E. cloacae complex	bla_{NDM}	10			10		
A. baumannii complex	bla_{NDM}	10			10		
E. aerogenes	bla_{IMP}	10	10				
P. aeruginosa	bla_{VIM}	6		2	9	2	1
P. aeruginosa	bla_{VIM}	6	9	1		3	1
P. aeruginosa	bla_{VIM}	7	1	1	2	2	7
K. pneumoniae	$bla_{OXA-48-like}$	10	10				
K. pneumoniae	$bla_{OXA-48-like}$	10	10				
K. pneumoniae	$bla_{OXA-48-like}$	10	9				1
E. aerogenes	Negative		1	10	9		
E. cloacae complex	Negative		2	10	3		5
E. cloacae complex	Negative		3	10	3		4
K. pneumoniae	Negative		1	10	9		

[a] The numbers represent the number of individual technologists scoring the reaction for the isolate as positive, negative, or indeterminate. n = 20 results per isolate, 10 for CIM and 10 for mHT.
[b] For the mHT, technologists marked "indeterminate" if they were not able to confidently score the isolate as positive or negative.

emase genes detected by the assay. Fifty isolates of CR-GNB that were negative for a carbapenemase gene (Table 1) also tested negative by the Xpert Carba-R assay. As expected, isolates that possessed other carbapenemases included in the CDC panel but not in the Xpert Carba-R assay (bla_{SME} and bla_{IMI}) tested negative by the Xpert Carba-R assay. Thus, the PPA and NPA for detection of bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{IMP}, and bla_{VIM} in bacterial isolates using the Xpert Carba-R are both 100%. The PPA and NPA for detection of any carbapenemase gene are 94.2% and 100.0%, respectively (Table 2).

CHROMOGENIC AGARS

Two chromogenic agars were evaluated in this study: HardyCHROM CRE and chromID CARBA agars. The agars were evaluated for recovery of meropenem-resistant organisms, and for specificity for recovery of CP-GNB. Both the *Enterobacteriaceae* and other GNB grew well on both agars (see online Supplemental Table 1). Overall, there was little difference between observations made at 24 h vs 48 h of incubation, with similarity between relative growth and color of the isolates at the different time points. Of the 2 *P. mirabilis* isolates included in this study, one (bla_{KPC} positive) did not grow on either agar and the other (bla_{NDM} positive) grew poorly (first quadrant only) on the HardyCHROM CRE but grew well on the chromID CARBA agar. The HardyCHROM CRE agar had more intraspecies color variation compared to the chromID CARBA agar (see online Supplemental Fig. 1). The colors produced by the organisms were as described in the product monograph for most isolates, although some variation did occur (see online Supplemental Fig. 2).

With the exception of 1 bla_{KPC}-positive *Proteus mirabilis* isolate that did not grow on either media type, all CP-GNB isolates grew on both agars. One $bla_{OXA-48-like}$–positive *Enterobacter aerogenes* grew only on the first quadrant on the chromID CARBA agar. For CR-GNB isolates that were negative for a carbapenemase gene,

53/58 isolates grew on the HardyCHROM CRE agar. The 5 CR-GNB isolates that either did not grow or grew poorly (first or second quadrant only) included 1 *Enterobacter cloacae*, 1 *Klebsiella pneumoniae*, and 3 *Serratia marcescens*. The chromID CARBA agar was more specific for growth of CP-GNB isolates. Twenty isolates of CR-GNB (3 *E. aerogenes*, 10 *E. cloacae*, 2 *K. pneumoniae*, 1 *Escherichia coli*, 1 *Hafnia alvei*, 1 *Klebsiella oxytoca*, 1 *Pseudomonas aeruginosa*, and 1 *Providencia rettgeri*) either failed to grow or grew poorly on the agar.

Discussion

Resistance to carbapenem antibiotics is considered an international public health threat. Throughout the world there are increasing cases of clinical infections with CP-GNB, including *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* complex. Herein, we have evaluated different methods for the screening and detection of CP-GNB. We examined 189 isolates previously characterized as meropenem-resistant, including 138 known carbapenemase-producing strains.

The CIM used herein is an adaptation of the method of van der Zwaluw et al. *(15)*. The adaptation improves the analytical performance characteristics of the assay, and also provides the ability to identify metallo-β-lactamase–producing strains. This method is a simple, inexpensive, and relatively rapid screen for CP-GNB that could be performed in any clinical microbiology laboratory. The modifications to the method of Zwaluw et al. include the use of TSB instead of water for the incubation step of the test bacteria isolate with the meropenem disk, and extension of the incubation time to 3–4 h as compared to the 2-h incubation time previously described. These modifications have the potential to increase the analytical sensitivity of the assay for isolates with weak carbapenemase activity.

The CIM uses materials commonly found in clinical laboratories (microcentrifuge tubes, meropenem disks, TSB, and Mueller–Hinton agar). Additionally, as demonstrated in the CIM to mHT comparison, this assay is less ambiguous to interpret compared to the mHT. For most *Enterobacteriaceae*, testing can be completed within 9 h with very little hands-on time (approximately 5 min). We used an overnight incubation for *P. aeruginosa* and *A. baumannii* complex since they were difficult to interpret at 6 h of incubation. Our comparison study between the CIM and mHT demonstrated that the CIM method is not only analytically more sensitive and specific than the mHT, but also has less interreader variability. The analytical specificity was especially improved for isolates of *Enterobacter* spp. since these isolates were likely AmpC hyperproducing strains, which have been previously described to cause false-positive results with the mHT *(22, 23)*.

Although the CIM demonstrated high overall percent agreement with the reference methods, 1 strain of *P. mirabilis* with a bla_{NDM} carbapenemase gave an ambiguous result because the zone around the meropenem disk was unable to be measured as a result of swarming of the *P. mirabilis* isolate. For nonfermenters, the growth of the carbapenem-susceptible *E. coli* surrounding the meropenem disks that were incubated with carbapenemase (bla_{VIM}) producing *P. aeruginosa* was not as robust as for the *Enterobacteriaceae* and required full overnight incubation for interpretation. As bla_{VIM} was the only carbapenemase gene in our collection of *P. aeruginosa* isolates, it is not clear if this growth characteristic would be present with other CR genes in *P. aeruginosa* or if it is a product of the bla_{VIM} determinant in this species. The 3 bla_{VIM}-positive *Enterobacteriaceae* isolates (2 *K. pneumoniae* and 1 *E. cloacae*) evaluated were unambiguous to interpret.

It is possible that the 7 "false-positives" by the CIM assay are actually CP-GNB strains possessing carbapenemase genes not detected by the PCR assays included in this study. Interestingly, we demonstrated that the *P. rettgeri* isolate and 5 of the 6 *A. baumannii* complex isolates tested were positive by CIM but negative by PCR. Using our CIM-EDTA assay, we confirmed that these isolates most likely encoded metallo-β-lactamases. These isolates were considered negative for the purposes of calculating the NPA; however, it is possible that these strains harbor uncommon or novel carbapenemase genes.

This study is among the first to evaluate the enhanced Xpert Carba-R assay for detection of carbapenemase genes in bacterial isolates; the US Food and Drug Administration recently cleared this assay *(24–29)*. The Xpert Carba-R assay demonstrated 100% PPA and NPA for the genetic targets represented in the assay. Prior evaluations of this method, albeit using an older version of the assay, demonstrated a decreased analytical sensitivity for the detection of $bla_{OXA\text{-}48\text{-}like}$–positive (especially OXA-181) isolates *(30–33)*. Since the discovery of $bla_{OXA\text{-}48}$, several variants have been identified that differ by only a few amino acid substitutions. $bla_{OXA\text{-}181}$, which is one of the most common variants, differs only by 4 amino acids from $bla_{OXA\text{-}48}$ *(34)*. Our study included 12 $bla_{OXA\text{-}48\text{-}like}$–positive isolates (11 *K. pneumoniae* and 1 *E. aerogenes*): 4 isolates were $bla_{OXA\text{-}181}$, 2 were $bla_{OXA\text{-}232}$, and 1 was a $bla_{OXA\text{-}162}$. Due to the previously reported false negatives for isolates with $bla_{OXA\text{-}48\text{-}like}$ genes, the assay was modified to increase sensitivity for detection of $bla_{OXA\text{-}48}$ variants. Consequently, our data and other recent studies evaluating modifications of the assay demonstrated increased sensitivity for $bla_{OXA\text{-}48\text{-}like}$–positive isolates *(24, 28, 35, 36)*.

In addition to testing for carbapenemase production, we also evaluated 2 chromogenic agars for their potential use in screening GNB isolates for carbapenemase production. The NPA for the detection of CP-

GNB was higher for the chromID CARBA agar (25.0%) as compared to the HardyCHROM CRE agar (0.0%) (Table 2). Both agars had growth of CR-GNBs, although the chromID CARBA agar had fewer breakthroughs of non–CP-GNB organisms (see online Supplemental Table 4). The superior performance of the chromID CARBA agar as compared to the HardyCHROM CRE agar to specifically recover CP-GNB was also demonstrated in a previous study *(20)*. As with the Xpert Carba-R assay, the detection of $bla_{\text{OXA-48-like}}$–positive isolates has been reported to be poor using previous formulations of chromogenic agars *(37–39)*. In our study, all 12 of the $bla_{\text{OXA-48-like}}$–positive isolates grew on both agars (third and fourth quadrants), although the *E. aerogenes* isolate only had growth in the first quadrant on the chromID CARBA agar.

Major strengths of this study include the large and diverse collection of isolates tested and the multiple methods of characterization performed, including a rapid and facile phenotypic method for carbapenemase detection from isolates of GNB. This study is limited by the evaluation of assay performance using only meropenem-resistant GNB; therefore, we had a high pretest probability for the detection of isolates possessing carbapenemase. Additionally, only 1 bla_{IMP}-positive isolate was evaluated in our study as a result of the scarcity of isolates with this genotype. At the time of writing, only 10 bla_{IMP}-positive isolates have been reported in the US *(40)*.

Overall, the CIM followed by the Cepheid Xpert Carba-R assay is an accurate method to detect and characterize CP-GNB, including *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* complex; this approach provides additional diagnostic tools for prompt recognition of these pathogens and improves both clinical outcomes and infection prevention efforts *(27)*.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: The US CDC provided the isolates in the Enterobacteriaceae Carbapenemase Diversity Panel that are part of the Antimicrobial Resistance strain bank. Cepheid provided the Xpert Carba-R cartridges used in this study. C.D. Burnham, Cepheid, bioMérieux, Theravance, and Accelerate Diagnostics.
Expert Testimony: None declared.
Patents: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, and final approval of manuscript.

Acknowledgments: We thank the US CDC for providing us with the isolates in the *Enterobacteriaceae* Carbapenemase Diversity Panel that are part of the Antimicrobial Resistance strain bank. These isolates significantly enriched this study. We thank Cepheid for providing the Xpert Carba-R cartridges used in this study.

References

1. CDC. Vital signs: carbapenem-resistant *Enterobacteriaceae*. MMWR Morb Mortal Wkly Rep 2013;62:165–70.
2. Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clin Microbiol Infect 2012;18:413–31.
3. Viau R, Frank KM. Intestinal carriage of carbapenemase-producing organisms: current status of surveillance methods. Clin Microbiol Rev 2016;29:1–27.
4. Hammoudi D, Moubareck CA, Sarkis DK. How to detect carbapenemase producers? A literature review of phenotypic and molecular methods. J Microbiol Methods 2014;107:106–18.
5. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 2007;20:440–58.
6. Poirel L, Pitout JD, Nordmann P. Carbapenemases: molecular diversity and clinical consequences. Future Microbiol 2007;2:501–12.
7. Falagas ME, Karageorgopoulos DE, Nordmann P. Therapeutic options for infections with *Enterobacteriaceae* producing carbapenem-hydrolyzing enzymes. Future Microbiol 2011;6:653–66.
8. Zasowski EJ, Rybak JM, Rybak MJ. The beta-lactams strike back: ceftazidime-avibactam. Pharmacotherapy 2015;35:755–70.
9. Castanheira M, Mills JC, Costello SE, Jones RN, Sader HS. Ceftazidime-avibactam activity tested against *Enterobacteriaceae* isolates from U.S. hospitals (2011 to 2013) and characterization of beta-lactamase-producing strains. Antimicrob Agents Chemother 2015;59:3509–17.
10. Vasoo S, Cunningham SA, Cole NC, Kohner PC, Menon SR, Krause KM, et al. In vitro activities of ceftazidime-avibactam, aztreonam-avibactam, and a panel of older and contemporary antimicrobial agents against carbapenemase-producing gram-negative bacilli. Antimicrob Agents Chemother 2015;59:7842–6.
11. Anderson KF, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, et al. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in *Enterobacteriaceae*. J Clin Microbiol 2007;45:2723–5.
12. CLSI. Performance standards for antimicrobial susceptibility testing, 26th ed. Wayne (PA): CLSI; 2016. CLSI supplement M100s.
13. CDC. FDA-CDC antimicrobial resistance isolate bank. http://www.cdc.gov/drugresistance/resistance-bank/currently-available.html (Accessed May 2016).
14. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, tenth ed. Wayne (PA): CLSI; 2015. CLSI document M01–A10.
15. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. 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 2015;10:e0123690.
16. Cepheid. GeneXpert Carba-R package insert, RCARBAR-10, Vol. Rev. 1 August 2015. 2015 ed [package insert]. Sunnyvale (CA): Cepheid: 2015.
17. Doern CD, Dunne WM, Burnham CA. Detection of Klebsiella pneumoniae carbapenemase (KPC) production in non-*Kebsiella pneumoniae Enterobacteriaceae* isolates by use of the Phoenix, Vitek 2, and disk diffusion methods. J Clin Microbiol 2011;49:1143–7.
18. CDC. Multiplex real-time PCR detection of *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamse (NDM-1). Atlanta (GA): CDC; 2010.
19. Mangold KA, Voss BL, Singh K, Thomson RB Jr, Schora DM, Peterson LR, Kaul KL. Multiple broad-spectrum beta-lactamase targets for comprehensive surveillance. J Clin Microbiol 2013;51:3423–5.
20. Pence MA, Hink T, Burnham CA. Comparison of chromogenic media for recovery of carbapenemase-producing enterobacteriaceae (CPE) and evaluation of CPE prevalence at a tertiary care academic medical center. J Clin Microbiol 2015;53:663–6.

21. US Food and Drug Administration. Statistical guidance on reporting results from studies evaluating diagnostic tests. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071148.htm (Accessed July 2016).

22. Mathers AJ, Carroll J, Sifri CD, Hazen KC. Modified Hodge test versus indirect carbapenemase test: prospective evaluation of a phenotypic assay for detection of *Klebsiella pneumoniae* carbapenemase (KPC) in *Enterobacteriaceae.* J Clin Microbiol 2013;51:1291–3.

23. Hung KH, Yan JJ, Lu JJ, Chen HM, Wu JJ. Characterization of the modified Hodge test-positive isolates of *Enterobacteriaceae* in Taiwan. J Microbiol Immunol Infect 2013;46:35–40.

24. Dortet L, Fusaro M, Naas T. Improvement of the Xpert Carba-R kit for the detection of carbapenemase-producing *Enterobacteriaceae.* Antimicrob Agents Chemother 2016;60:3832–7.

25. Kim DK, Kim HS, Pinto N, Jeon J, D'Souza R, Kim MS, et al. Xpert Carba-R assay for the detection of carbapenemase-producing organisms in intensive care unit patients of a Korean tertiary care hospital. Ann Lab Med 2016;36:162–5.

26. Uwamino Y, Sugita K, Hasegawa N, Nishimura T, Fujiwara H, Iwata S. Rapid detection and typing of carbapenemase-producing genes from carbapenem-resistant *Enterobacteriaceae* isolates collected in a Japanese hospital using the Xpert Carba-R assay. Jpn J Infect Dis [Epub ahead of print 2016 Aug 31].

27. Cortegiani A, Russotto V, Graziano G, Geraci D, Saporito L, Cocorullo G, et al. Use of Cepheid Xpert Carba-R for rapid detection of carbapenemase-producing bacteria in abdominal septic patients admitted to intensive care unit. PloS One 2016;11:e0160643.

28. Tato M, Ruiz-Garbajosa P, Traczewski M, Dodgson A, McEwan A, Humphries R, et al. Multisite evaluation of Cepheid Xpert Carba-R assay for detection of carbapenemase-producing organisms in rectal swabs. J Clin Microbiol 2016;54:1814–9.

29. Smith M, Diederen B, Scahrringa J, Leversteijn-van Hall M, Fluit AC, Cohen Stuart J. Rapid and accurate detection of carbapenemase genes in *Enterobacteriaceae* with the Cepheid Xpert Carba-R assay. J Med Microbiol 2016;65:951–3.

30. Decousser JW, Poirel L, Desroches M, Jayol A, Denamur E, Nordmann P. Failure to detect carbapenem-resistant *Escherichia coli* producing OXA-48-like using the Xpert Carba-R assay. Clin Microbiol Infect 2015;21:e9–10.

31. Lafeuille E, Laouira S, Sougakoff W, Soulier-Escrihuela O, Leconte J, Garrec H, et al. Detection of OXA-48-like carbapenemase genes by the Xpert Carba-R test: room for improvement. Int J Antimicrob Agents 2015;45:441–2.

32. Anandan S, Damodaran S, Gopi R, Bakthavatchalam YD, Veeraraghavan B. Rapid screening for carbapenem resistant organisms: current results and future approaches. J Clin Diagn Res 2015;9:DM01–3.

33. Boo TW, McGrath E, Davitt J, Grogan J, O'Sullivan N, Hopkins KL, et al. Cross-transmission of *Escherichia coli* producing OXA-181 in hospitalized patients and failure of carbapenemase detection by commercial and in-house PCR assays. J Med Microbiol 2016;65:99–100.

34. Bakthavatchalam YD, Anandan S, Veeraraghavan B. Laboratory detection and clinical implication of oxacillinase-48 like carbapenemase: the hidden threat. J Glob Infect Dis 2016;8:41–50.

35. Genc O, Aksu E, Gulcan A. The identification of carbapenemase types in *Enterobacteriaceae* by using molecular assay and phenotyping confirmation tests. J Microbiol Methods 2016;125:8–11.

36. Findlay J, Hopkins KL, Meunier D, Woodford N. Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. J Antimicrob Chemother 2015;70:1338–42.

37. Wilkinson KM, Winstanley TG, Lanyon C, Cummings SP, Raza MW, Perry JD. Comparison of four chromogenic culture media for carbapenemase-producing *Enterobacteriaceae.* J Clin Microbiol 2012;50:3102–4.

38. Zarakolu P, Day KM, Sidjabat HE, Kamolvit W, Lanyon CV, Cummings SP, et al. Evaluation of a new chromogenic medium, chromID OXA-48, for recovery of carbapenemase-producing *Enterobacteriaceae* from patients at a university hospital in Turkey. Eur J Clin Microbiol Infect Dis 2015;34:519–25.

39. Girlich D, Anglade C, Zambardi G, Nordmann P. Comparative evaluation of a novel chromogenic medium (chromID OXA-48) for detection of OXA-48 producing *Enterobacteriaceae.* Diagn Microbiol Infect Dis 2013;77:296–300.

40. CDC. Healthcare-associated infections: tracking CRE. http://www.cdc.gov/hai/organisms/cre/TrackingCRE.html (Accessed May 2016).