

Washington University School of Medicine

Digital Commons@Becker

Open Access Publications

6-24-2020

Evaluation of NG-Test Carba 5 for rapid phenotypic detection and differentiation of five common carbapenemase families: Results of a multicenter clinical evaluation

Stephen Jenkins

Carey-Ann D. Burnham


Meghan A Wallace

et al

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs



Evaluation of NG-Test Carba 5 for Rapid Phenotypic Detection and Differentiation of Five Common Carbapenemase Families: Results of a Multicenter Clinical Evaluation

Stephen Jenkins,^a Nathan A. Ledebor,^b Lars F. Westblade,^a  Carey-Ann D. Burnham,^d Matthew L. Faron,^b Yehudit Bergman,^e Rebecca Yee,^e Brian Mesich,^b Derek Gerstbrein,^b Meghan A. Wallace,^d Amy Robertson,^a Kathy A. Fautleroy,^a Anna S. Klavins,^c Rianna Malherbe,^c Andre Hsiung,^c Patricia J. Simner^e

^aWeill Cornell Medicine, New York, New York, USA

^bMedical College of Wisconsin, Milwaukee, Wisconsin, USA

^cHardy Diagnostics, Santa Maria, California, USA

^dWashington University in St. Louis School of Medicine, St. Louis, Missouri, USA

^eJohns Hopkins University School of Medicine, Baltimore, Maryland, USA

Stephen Jenkins, Nathan A. Ledebor, Lars F. Westblade, Carey-Ann D. Burnham, and Patricia J. Simner contributed equally to this work. Author order was determined on the basis of being identified as a site principal investigator and the order the site was enrolled in the study.

ABSTRACT NG-Test Carba 5 is a rapid *in vitro* multiplex immunoassay for the phenotypic detection and differentiation of five common carbapenemase families (KPC, OXA-48-like, VIM, IMP, and NDM) directly from bacterial colonies. The assay is simple to perform and has recently received U.S. Food and Drug Administration clearance. A method comparison study was performed at geographically diverse medical centers ($n = 3$) in the United States, where 309 *Enterobacterales* and *Pseudomonas aeruginosa* isolates were evaluated by NG-Test Carba 5 (NG Biotech, Guipry, France), the Xpert Carba-R assay (Cepheid, Inc., Sunnyvale, CA), the modified carbapenem inactivation method (mCIM), the EDTA-modified carbapenem inactivation method, and disk diffusion with carbapenems. Colonies from tryptic soy agar with 5% sheep blood (blood agar) and MacConkey agar were tested, and the results were compared to those obtained by a composite reference method. Additionally, a fourth medical center performed a medium comparison study by evaluating the performance characteristics of NG-Test Carba 5 from blood, MacConkey, and Mueller-Hinton agars with 110 isolates of *Enterobacterales* and *P. aeruginosa*. These results were compared to the expected genotypic and mCIM results. For the multicenter method comparison study, the overall positive percent agreement (PPA) and the overall negative percent agreement (NPA) of NG-Test Carba 5 with the composite reference method were 100% for both blood and MacConkey agars. The medium comparison study at the fourth site showed that the PPA ranged from 98.9% to 100% and that the NPA ranged from 95.2% to 100% for blood, MacConkey, and Mueller-Hinton agars. NG-Test Carba 5 accurately detected and differentiated five common carbapenemase families from *Enterobacterales* and *P. aeruginosa* colonies on commonly used agar media. The results of this test will support a streamlined laboratory work flow and will expedite therapeutic and infection control decisions.

KEYWORDS *Enterobacterales*, NG-Test Carba 5, *Pseudomonas aeruginosa*, Xpert Carba-R, carbapenemase, eCIM, mCIM

According to the Centers for Disease Control and Prevention (CDC), carbapenem-resistant *Enterobacterales* and carbapenem-resistant *Pseudomonas aeruginosa* are considered urgent and serious threats, respectively, in the United States (1–3). Mech-

Citation Jenkins S, Ledebor NA, Westblade LF, Burnham C-AD, Faron ML, Bergman Y, Yee R, Mesich B, Gerstbrein D, Wallace MA, Robertson A, Fautleroy KA, Klavins AS, Malherbe R, Hsiung A, Simner PJ. 2020. Evaluation of NG-Test Carba 5 for rapid phenotypic detection and differentiation of five common carbapenemase families: results of a multicenter clinical evaluation. *J Clin Microbiol* 58:e00344-20. <https://doi.org/10.1128/JCM.00344-20>.

Editor Daniel J. Diekema, University of Iowa College of Medicine

Copyright © 2020 American Society for Microbiology. All Rights Reserved.

Address correspondence to Patricia J. Simner, psimner1@jhmi.edu.

Received 25 February 2020

Returned for modification 23 March 2020

Accepted 28 April 2020

Accepted manuscript posted online 6 May 2020

Published 24 June 2020

organisms of resistance among carbapenem-resistant organisms (CRO) are broadly divided into 2 groups: (i) carbapenemase-producing CRO (CP-CRO) and (ii) non-CP-CRO; i.e., CRO resistant to carbapenems due to non-carbapenemase-mediated mechanisms, such as membrane permeability defects in combination with extended-spectrum β -lactamase (ESBL) or AmpC β -lactamase production. The former are more aggressively targeted by infection control and antimicrobial stewardship teams due to the ease of transmission of carbapenemase genes among Gram-negative bacteria through the horizontal transfer of plasmids, which often contain additional antimicrobial resistance determinants that further limit treatment options (4–8). Moreover, infections caused by CP-CRO are associated with increased mortality compared to those caused by non-CP-CRO (5, 9–11). Thus, carbapenemase detection among CRO has become increasingly important in recent years for patient care, public health, and infection control initiatives.

The detection and differentiation of carbapenemases from cultured isolates in clinical and public health laboratories normally involve the initial detection of decreased susceptibility to carbapenems followed by the broad detection of carbapenemase production by a phenotypic method (e.g., a carbapenem hydrolysis assay, such as CarbaNP, or the modified carbapenem inactivation method [mCIM]) and/or detection of specific carbapenemase genes by molecular-based assays (e.g., the Xpert Carba-R assay [Cepheid, Inc., Sunnyvale, CA]) (12, 13). Although there has been significant progress over the past decade in developing phenotypic assays with improved performance characteristics and rapid, sample-to-answer molecular approaches, there are still limitations to these methods, such as the inability to detect all carbapenemase variants, labor intensity, work flow, turnaround time, and cost (12, 13).

NG-Test Carba 5 (NG Biotech, Guipry, France) is an immunoassay intended to streamline the process of carbapenemase detection and differentiation in routine clinical laboratories. It is a rapid diagnostic test (≤ 15 min) based on the immunochromatographic detection of the five most common carbapenemase families (KPC, OXA-48-like, VIM, IMP, and NDM) directly from bacterial colonies. Within the OXA-48-like family, NG-Test Carba 5 has demonstrated inclusivity with at least 15 different confirmed variants, including OXA-163, OXA-181, and OXA-232 (14). The purpose of this multicenter study was to establish the performance characteristics of the NG-Test Carba 5 test from tryptic soy agar with 5% sheep blood (blood agar) and MacConkey agar to obtain U.S. Food and Drug Administration (FDA) clearance of NG-Test Carba 5 for *in vitro* diagnostic use as well as to evaluate its performance from Mueller-Hinton agar compared to that from blood and MacConkey agars.

MATERIALS AND METHODS

Bacterial isolates. Testing was performed at three academic medical centers in the United States, including the Johns Hopkins University School of Medicine (site 1; Baltimore, MD), Weill Cornell Medicine (site 2; New York, NY), and the Medical College of Wisconsin (site 3; Milwaukee, WI). Each site had to pass a proficiency testing panel prior to initiation of the study.

Retrospective and prospective isolates were included in the study, for a total of 309 *Enterobacteriales* isolates ($n = 240$) and *P. aeruginosa* isolates ($n = 69$) (see Table S1 in the supplemental material). The 121 retrospective challenge isolates were distributed among the three clinical sites and consisted of reference bank isolates (American Type Culture Collection, National Collection of Type Cultures, CDC, International Health Management Associates, JMI Laboratories, Laboratory Specialists, Inc.) and clinical isolates from California hospitals and the University of Illinois.

Each clinical site contributed its own retrospective isolates (collected >6 months from the testing date [$n = 38$]) and prospective isolates (collected <6 months from the testing date [$n = 150$]) for the remaining 188 clinical isolates (Table S1). The inclusion criteria included any *Enterobacteriales* or *P. aeruginosa* isolate identified by standard-of-care testing and included isolates that were susceptible or not susceptible to a carbapenem(s) or that contained a previously determined mechanism of carbapenem resistance. The prospective isolates were collected from various specimen types (e.g., urine, rectal swabs/stools, blood, respiratory specimens, wounds, sterile fluids, tissues, etc.).

All isolates were subcultured twice prior to testing. Isolates recovered from frozen stocks were streaked onto blood agar with an ertapenem disk placed (including *P. aeruginosa* isolates) between the 3rd and 4th quadrants and incubated at $35 \pm 2^\circ\text{C}$ overnight. After overnight incubation, growth selected from around the ertapenem disk was streaked from the first subculture to blood agar and MacConkey agar plates with an ertapenem disk placed between the 3rd and 4th quadrants and incubated at $35 \pm 2^\circ\text{C}$ for 18 to 24 h.

TABLE 1 Pre-discrepant analysis result interpretation of NG-Test Carba 5 compared to the composite reference method

Composite reference method result		NG-Test Carba 5 result	Pre-discrepant NG-Test Carba 5 interpretation	No. of results by pre-discrepant analysis ^a
Xpert Carba-R	mCIM ^b			
+	+	+	True positive	169
–	–	+	False positive	0
+	–	+	False positive	0
–	+	+	False positive	7 ^c
+	+	–	False negative	0
+	–	–	True negative (lack of or low-level expression)	1 ^d
–	+	–	True negative (other off-target carbapenemase)	15 ^e
–	–	–	True negative	121 ^f

^aThe results on blood and MacConkey agars were the same. Results are shown by enzyme type and not by isolate. The 172 on-target carbapenemase-producing isolates demonstrated a total of 176 positive NG-Test Carba 5 results due to four isolates that coproduced two target carbapenemases.

^bmCIM, modified carbapenem inactivation method.

^cOf the 7 false-positive results, results for IMP for 6 isolates (3 *Enterobacteriales* and 3 *Pseudomonas aeruginosa* isolates) were positive by NG-Test Carba 5 and negative by Xpert Carba-R. The remaining false-positive result was for a *Klebsiella oxytoca* isolate that was positive for both KPC and NDM by NG-Test Carba 5 but negative for NDM by Xpert Carba-R. PCR and bidirectional sequencing confirmed all 7 NG-Test Carba 5 results.

^dOne *Enterobacter cloacae* prospective isolate was resistant to ertapenem (zone diameter, 18 mm) but susceptible to imipenem and meropenem and harbored the *bla*_{KPC} gene as determined by Xpert Carba-R. The isolate was negative by both mCIM and NG-Test Carba 5, indicating a lack of or low-level expression.

^eThese 15 true-negative results were found for 7 *Enterobacteriales* isolates and 8 *P. aeruginosa* isolates. Of the *Enterobacteriales* isolates, four harbored *bla*_{SME}. A carbapenemase gene was not detected in the remaining three isolates, and these isolates likely had false-positive mCIM results due to ESBL and/or AmpC expression combined with permeability defects. Among the *P. aeruginosa* isolates, one harbored the *bla*_{GES} gene and the remaining seven harbored non-carbapenemase β -lactamase genes.

^fOne *P. aeruginosa* isolate and one *Klebsiella aerogenes* isolate led to indeterminate mCIM results and were negative by Xpert Carba-R and NG-Test Carba 5.

NG-Test Carba 5. Blood and MacConkey agar overnight cultures were tested with NG-Test Carba 5 following the manufacturer's instructions. Using a 1- μ l loop, three colonies were touched and inoculated into a 1.5-ml microcentrifuge tube containing 5 drops of extraction buffer. After the buffer was inoculated with colonies, the tube was vortexed for approximately 3 to 5 s. Mucoid, or so-called sticky, colonies required a longer vortex time of approximately 10 to 15 s. Using a small transfer pipette provided in the NG-Test Carba 5 kit, 100 μ l of the suspension was inoculated into the NG-Test Carba 5 sample well. After 15 min, the test was visually examined for the presence or absence of the control and test lines. To avoid biased result interpretation, NG-Test Carba 5 results were visually examined for isolates grown on blood and MacConkey agars by separate study team members. Quality control (QC) was performed every day of testing and included a negative control (*Klebsiella pneumoniae* ATCC BAA-1706) and one positive control for each target (KPC-producing *K. pneumoniae* ATCC BAA-1705, OXA-48-producing *K. pneumoniae* NCTC 13442, VIM-producing *K. pneumoniae* NCTC 13439, IMP-producing *Escherichia coli* NCTC 13476, and NDM-producing *K. pneumoniae* ATCC BAA-2146).

Composite reference method. The NG-Test Carba 5 assay was compared to a composite reference method. The composite reference method included (i) phenotypic detection of carbapenemase production by mCIM and (ii) molecular detection of carbapenemase genes by the U.S. FDA-cleared Xpert Carba-R real-time PCR assay (1, 12, 13, 15, 17–22). Table 1 summarizes how the NG-Test Carba 5 results were interpreted based on the results of the composite reference method prior to discrepant analysis. The composite reference method was chosen because NG-Test Carba 5 detects the carbapenemase enzymes themselves and the mCIM is necessary to determine the expression of the gene product detected by Xpert Carba-R. In addition to the composite reference standard, carbapenem (ertapenem [10 μ g] for *Enterobacteriales* only, imipenem [10 μ g], and meropenem [10 μ g]) antimicrobial susceptibility testing (AST) by disk diffusion and the EDTA-modified carbapenem inactivation method (eCIM) were performed, and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (1, 19–22). All comparator methods were performed from the blood agar plate. Positive and negative controls for Xpert Carba-R, disk diffusion, mCIM, and eCIM were performed on each day of testing following manufacturer or CLSI guidelines, as appropriate (1, 18–22).

Discrepant analysis. Organisms that had discrepant NG-Test Carba 5 and composite reference method results were further analyzed by targeted PCR and sequencing by an independent reference laboratory to confirm the presence or absence of the carbapenemase genotype and variant (Table 1). Isolates were grown overnight at 35 \pm 2°C on blood agar, and DNA was purified using a QIAcube instrument following the recommendation of the manufacturer (Qiagen, Gaithersburg, MD). The *Enterobacteriales* isolates were screened for the presence of the carbapenemase families *bla*_{KPC}, *bla*_{OXA-48}, *bla*_{IMP},

*bla*_{VIM}, *bla*_{NDM}, and *bla*_{GES} by multiplex PCR using published primers (23, 24). The *P. aeruginosa* isolates were also screened for *bla*_{SPM} and *bla*_{GIM} (23, 24). β -Lactamase genes were amplified and sequenced in their entirety. The amino acid sequence was compared to the sequences available in databases maintained by the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov) to identify enzyme variants. Whole-genome sequencing was performed on an Illumina (Illumina, San Diego, CA) and/or Nanopore (Oxford Nanopore Technologies, Oxford, England) sequencing platform to elaborate upon any further discrepant results.

Additional medium comparison study. A secondary study (separate from the multicenter clinical trial) was performed. A fourth site (site 4; Washington University, St. Louis, MO) evaluated the performance of NG-Test Carba 5 from isolates grown on blood, MacConkey, and Mueller-Hinton agars and compared the results to the expected molecular and mCIM results for 110 previously molecularly characterized *Enterobacteriales* isolates ($n = 105$) and *P. aeruginosa* isolates ($n = 5$). Sixty of these organisms were a subset of the retrospective challenge isolates that were supplied by Hardy Diagnostics (Santa Maria, CA) and were also evaluated by each of the three clinical trial sites (sites 1 to 3). The remaining 50 isolates were from the Washington University strain bank (Table S2). Of these, 34 originated from Barnes-Jewish Hospital and 16 were from Pakistan (hospital environmental isolates or isolates recovered from urine). Each isolate had a genotypic resistance mechanism previously determined using molecular methods (targeted PCR, Xpert Carba-R, or whole-genome sequencing). Primary cultures were prepared by streaking all isolates to blood agar with an ertapenem disk placed between the 3rd and 4th quadrants. All isolates (including *P. aeruginosa* isolates) were subsequently streaked to blood and MacConkey agars with an ertapenem disk placed between the 3rd and 4th quadrants. Disk diffusion was performed, and the result for each organism with ertapenem on Mueller-Hinton agar was interpreted following CLSI guidelines (19, 20). Three colonies (blood and MacConkey agars) or spots (Mueller-Hinton agar) around the ertapenem disk were touched with a 1- μ l loop and inoculated into the extraction buffer before completing the NG-Test Carba 5 procedure.

Statistical analysis. All data were entered into and analyzed in Microsoft Excel software for positive percent agreement (PPA) and negative percent agreement (NPA). Upper- and lower-bound 95% confidence intervals (CIs) were calculated and are shown in the appropriate tables (25, 26).

RESULTS

NG-Test Carba 5 versus composite reference method. The pre-discrepant analysis NG-Test Carba 5 results versus the results of the composite reference method are summarized in Table 1. The overall pre-discrepant analysis PPA was 100% (95% confidence interval [CI], 97.8% to 100.0%), and the NPA was 95.1% (95% CI, 90.3% to 97.6%), regardless of medium type. Prior to discrepant analysis, there were seven false-positive results associated with four *Enterobacteriales* and three *P. aeruginosa* isolates (Table 2). The results for the seven isolates were concordant between NG-Test Carba 5 and mCIM but were negative by the Xpert Carba-R assay.

Among the *Enterobacteriales*, three of the isolates (two *Enterobacter cloacae* isolates, one *Serratia marcescens* isolate) were considered false positive for IMP by NG-Test Carba 5. Targeted PCR and bidirectional sequencing revealed that these three isolates were confirmed to harbor the *bla*_{IMP-8} gene, in favor of the NG-Test Carba 5 result. Interestingly, *bla*_{IMP-8} is predicted to be detected by Xpert Carba-R, based on *in silico* analysis, but this has not been tested analytically by Cepheid (16). The fourth isolate, *Klebsiella oxytoca*, was considered to have a true-positive result for KPC and a false-positive result for NDM, based on the composite reference standard (Table 2). This isolate was later confirmed by PCR and bidirectional sequencing to coproduce *bla*_{KPC} and *bla*_{NDM-1}.

Three *P. aeruginosa* isolates were false positive for IMP by NG-Test Carba 5 but negative by Xpert Carba-R (Table 2). All three were positive by mCIM and confirmed to have a *bla*_{IMP} gene by PCR and sequencing (*bla*_{IMP-7}, *bla*_{IMP-15}, *bla*_{IMP-19}). Of note, *bla*_{IMP-7} and *bla*_{IMP-15} are not predicted to be detected by Xpert Carba-R, while *bla*_{IMP-19} is predicted to be detected based on *in silico* analysis but has not been tested analytically by the manufacturer (16, 18).

Table 3 summarizes the post-discrepant analysis of NG-Test Carba 5 performance versus the composite comparator method. The overall PPA was 100% (95% CI, 97.8% to 100%) and the overall NPA was 100% (95% CI, 97.3% to 100%), regardless of the organism group or agar medium used.

Carbapenem susceptibility testing. Of the 240 *Enterobacteriales* isolates that were included in the analysis (Table 3), 196 (81.7%) were not susceptible to a carbapenem (intermediate or resistant). Of the *Enterobacteriales* isolates, 79.2% (190/240), 73.8% (177/240), and 74.2% (178/240) were not susceptible to ertapenem, imipenem, and meropenem, respectively. Among the 69 *P. aeruginosa* isolates, 64 (92.8%) were not

TABLE 2 Summary of discrepant NG-Test Carba 5 results^a

Site	Organism identification	Disk diffusion results						NG-Test Carba 5 result on:				Xpert Carba-R result	Alternative PCR + sequencing result	Final interpretation post-discrepant analysis		
		ETP		IPM		MEM		Blood agar	MacConkey agar	eCIM	mCIM					
		ZD (mm)	Interpretation	ZD (mm)	Interpretation	ZD (mm)	Interpretation								ZD (mm)	Result
1	<i>Enterobacter cloacae</i>	16	R	13	R	18	R	6	Pos	6	Neg	IMP	IMP	Neg	IMP-8	True positive
2	<i>Enterobacter cloacae</i>	17	R	19	R	19	R	6	Pos	6	Neg	IMP	IMP	Neg	IMP-8	True positive
3	<i>Serratia marcescens</i>	6	R	6	R	9	R	6	Pos	19	Pos	IMP	IMP	Neg	IMP-8	True positive
3	<i>Klebsiella oxytoca</i>	10	R	6	R	10	R	6	Pos	6	Neg	KPC, NDM	KPC, NDM	KPC	KPC, NDM	True positive
1	<i>Pseudomonas aeruginosa</i>	NA	NA	16	R	6	R	NA	Pos	NA	NA	IMP	IMP	Neg	IMP-7	True positive
3	<i>Pseudomonas aeruginosa</i>	NA	NA	6	R	6	R	6	Pos	6	Pos	IMP	IMP	Neg	IMP-15	True positive
2	<i>Pseudomonas aeruginosa</i>	NA	NA	11	R	6	R	6	Pos	6	Pos	IMP	IMP	Neg	IMP-19	True positive

^aZD, disk diffusion zone diameter; R, resistant; ETP, ertapenem; IPM, imipenem; MEM, meropenem; eCIM, EDTA-modified carbenem inactivation method; mCIM, modified carbenem inactivation method; Neg, negative; Pos, positive; NA, not applicable.

TABLE 3 Composite reference method versus NG-Test Carba 5 results by isolate tested from blood or MacConkey agar, post-discrepant analysis^a

Organism (total no. of isolates)	NG-Test Carba 5 carbapenemase target (no. of expected positives)	No. of results				PPA	95% CI		NPA	95% CI	
		TP	FP	FN	TN		Low	High		Low	High
<i>Enterobacteriales</i> (240)	KPC (<i>n</i> = 83)	83	0	0	157	100.0	95.6	100.0	100.0	97.6	100.0
	OXA-48-like (<i>n</i> = 17)	17	0	0	223	100.0	81.6	100.0	100.0	98.3	100.0
	VIM (<i>n</i> = 10)	10	0	0	230	100.0	72.3	100.0	100.0	98.4	100.0
	IMP (<i>n</i> = 7)	7	0	0	233	100.0	64.6	100.0	100.0	98.4	100.0
	NDM (<i>n</i> = 35)	35	0	0	205	100.0	90.1	100.0	100.0	98.2	100.0
	OXA-48 + NDM (<i>n</i> = 2)	2	0	0	238	100.0	34.2	100.0	100.0	98.4	100.0
	OXA-48 + VIM (<i>n</i> = 1)	1	0	0	239	100.0	20.7	100.0	100.0	98.4	100.0
	KPC + NDM (<i>n</i> = 1)	1	0	0	239	100.0	20.7	100.0	100.0	98.4	100.0
Overall (<i>n</i> = 156)	156	0	0	84	100.0	97.6	100.0	100.0	95.6	100.0	
<i>Pseudomonas aeruginosa</i> (69)	KPC (<i>n</i> = 2)	2	0	0	67	100.0	34.2	100.0	100.0	94.6	100.0
	OXA-48-like (<i>n</i> = 0)	0	0	0	69	NA	NA	NA	100.0	94.7	100.0
	VIM (<i>n</i> = 9)	9	0	0	60	100.0	70.1	100.0	100.0	94.0	100.0
	IMP (<i>n</i> = 5)	5	0	0	64	100.0	56.6	100.0	100.0	94.3	100.0
	NDM (<i>n</i> = 0)	0	0	0	69	NA	NA	NA	100.0	94.7	100.0
	Overall (<i>n</i> = 16)	16	0	0	53	100.0	80.6	100.0	100.0	93.2	100.0
<i>Enterobacteriales</i> + <i>P. aeruginosa</i> (309)	Overall (<i>n</i> = 172)	172	0	0	137	100.0	97.8	100.0	100.0	97.3	100.0

^aThe results on blood and MacConkey agars were the same. Testing was performed at sites 1 to 3. TP, true positive; FP, false positive; TN, true negative; FN, false negative; NA, not applicable; 95% CI, 95% confidence interval.

susceptible to a carbapenem. Of those, 81.2% (56/69) and 92.8% (64/69) were not susceptible to imipenem and meropenem, respectively.

mCIM and eCIM analysis. Table 4 shows the agreement of the NG-Test Carba 5 results with the mCIM and eCIM results. When the mCIM and eCIM results were compared to the NG-Test Carba 5 results for *Enterobacteriales*, the overall PPA was 93.6% (95% CI, 87.3% to 96.9%) for serine β -lactamase detection and 94.2% (95% CI, 84.4% to 98.0%) for metallo- β -lactamase (MBL) detection. The overall NPA was 100%. Seven *Enterobacteriales* isolates showed a positive mCIM result and a negative NG-Test Carba 5 result. Four *S. marcescens* isolates were positive for the carbapenemase gene *bla*_{SME}. A carbapenemase gene was not detected in the remaining three isolates, and these likely had false-positive mCIM results due to ESBL and/or AmpC expression combined with permeability defects. Four organisms coproduced a serine carbapenemase and MBL (KPC plus NDM [*n* = 1], OXA-48 plus NDM [*n* = 2], OXA-48 plus VIM [*n* = 1]); in these instances, the serine β -lactamase masked the presence of the MBL, resulting in a false-negative eCIM result.

The overall PPA for NG-Test Carba 5 compared to mCIM for both *Enterobacteriales* and *P. aeruginosa* was 92% (95% CI, 87.2% to 95.1%), and the NPA was 100% (95% CI, 96.9% to 100%). Among the *P. aeruginosa* isolates, there were eight with mCIM-positive results that were negative by NG-Test Carba 5. One *P. aeruginosa* isolate harbored the *bla*_{GES} gene. The remaining seven *P. aeruginosa* isolates harbored noncarbapenemase β -lactamase genes, including *bla*_{OXA-2} (*n* = 5) and *bla*_{OXA-10} (*n* = 1), and all of the isolates carried the chromosomal β -lactamase genes *bla*_{OXA-50} and *bla*_{PAO} (*n* = 7). These results may explain the positive mCIM results since the original multicenter mCIM evaluation identified a false-positive *P. aeruginosa* isolate that coharbored the β -lactamase genes *bla*_{OXA-2}, *bla*_{OXA-50}, and *bla*_{PAO} (1).

Additional medium comparison study. Table 5 shows the performance results for NG-Test Carba 5 by agar type (site 4). The PPA for *Enterobacteriales* was 100% (95% CI, 95.7% to 100%), 100% (95% CI, 95.7% to 100%), and 98.8% (95% CI, 93.6% to 99.8%) for blood, MacConkey, and Mueller-Hinton agars, respectively. The NPA for *Enterobacteriales* was 100% (95% CI, 80.6% to 100%), 93.8% (95% CI, 71.7% to 98.9%), and 100% (95% CI, 80.6% to 100%) for blood, MacConkey, and Mueller-Hinton agars, respectively. One *Providencia rettgeri* isolate from the Washington University strain bank was false

TABLE 4 NG-Test Carba 5 agreement with mCIM and eCIM by isolate from three clinical sites, pre-discrepant analysis^a

Test, organism group ^b	mCIM or mCIM + eCIM result	No. of isolates	NG-Test Carba 5 ^c		Agreement
			Result	No. of isolates	
mCIM					
<i>Enterobacteriales</i>	Positive	163	KPC OXA-48-like VIM IMP NDM OXA-48 + VIM OXA-48 + NDM KPC + NDM Negative	83 17 10 7 35 1 2 1 7 ^d	PPA, 156/163 = 95.7% (95% CI, 91.4–97.9%)
	Negative	76	Negative	76	NPA, 76/76 = 100% (95% CI, 95.2–100.0%)
<i>P. aeruginosa</i>	Positive	24	KPC OXA-48-like VIM IMP NDM Negative	2 0 9 5 0 8 ^e	PPA, 16/24 = 66.7% (95% CI, 46.7–82.0%)
	Negative	44	Negative	44	NPA, 44/44 = 100% (95% CI, 92.1–100.0%)
<i>Enterobacteriales</i> + <i>P. aeruginosa</i>	Positive	187	KPC OXA-48-like VIM IMP NDM OXA-48 + VIM OXA-48 + NDM KPC + NDM Negative	85 17 19 12 35 1 2 1 15 ^{d,e}	PPA, 172/187 = 92.0% (95% CI, 87.2–95.1%)
	Negative	120	Negative	120	NPA, 120/120 = 100% (95% CI, 96.9–100.0%)
mCIM + eCIM, <i>Enterobacteriales</i>					
	Serine β-lactamase	112	KPC OXA-48-like VIM IMP NDM OXA-48 + VIM OXA-48 + NDM KPC + NDM Negative	83 15 0 2 ^f 1 ^f 1 2 1 7 ^d	PPA, 102/112 = 93.6% (95% CI, 87.3–96.9%)
	MBL	51	KPC OXA-48-like VIM IMP NDM OXA-48 + VIM OXA-48 + NDM KPC + NDM Negative	0 2 ^g 10 5 34 0 0 0 0	PPA, 49/51 = 94.2% (95% CI, 84.4–98.0%)
	Negative	76	Negative	76	NPA, 76/76 = 100% (95% CI, 95.2–100.0%)

^aTesting was performed at sites 1 to 3. mCIM, modified carbapenem inactivation method; eCIM, EDTA-modified carbapenem inactivation method.
^bOne *K. aerogenes* isolate and one *P. aeruginosa* isolate had an indeterminate mCIM result and were NG-Test Carba 5 negative. These results are not shown here.
^cThe NG-Test Carba 5 result for each isolate was the same from blood and MacConkey agars.
^dFour *S. marcescens* isolates were positive for the carbapenemase gene *bla*_{SME}. A carbapenemase gene was not detected in the remaining three isolates, and these isolates were likely false positive due to ESBL and/or AmpC expression combined with permeability defects.
^eOne *P. aeruginosa* isolate had a *bla*_{GES} gene. Seven *P. aeruginosa* isolates were confirmed to harbor noncarbapenemase β-lactamase genes by whole-genome sequencing, likely indicating false-positive mCIM results.
^fUpon retesting by mCIM and eCIM, a positive result for MBL was obtained for one of the two IMP isolates and the NDM isolate.
^gUpon retesting by mCIM and eCIM, a positive result for serine β-lactamase was obtained for one of the two OXA-48 isolates. OXA-48-producing isolates are known to produce false-positive eCIM results.

TABLE 5 NG-Test Carba 5 medium comparison study by isolate, pre-discrepant analysis^a

Plate	Organism group (total no. of isolates)	NG-Test Carba 5 carbapenemase target (no. of expected positives)	No. of results				95% CI			95% CI			
			TP	FP	FN	TN	PPA	Low	High	NPA	Low	High	
Blood agar	<i>Enterobacteriales</i> (101)	KPC (<i>n</i> = 26)	26	0	0	75	100.0	87.1	100.0	100.0	95.1	100.0	
		OXA-48-like (<i>n</i> = 15)	15	0	0	86	100.0	79.6	100.0	100.0	95.7	100.0	
		VIM (<i>n</i> = 7)	7	0	0	94	100.0	64.6	100.0	100.0	96.1	100.0	
		IMP (<i>n</i> = 6)	6	0	0	95	100.0	61.0	100.0	100.0	96.1	100.0	
		NDM (<i>n</i> = 27)	27	0	0	74	100.0	87.5	100.0	100.0	95.1	100.0	
		OXA-48 + NDM (<i>n</i> = 3)	3	0	0	98	100.0	43.9	100.0	100.0	96.2	100.0	
		OXA-48 + VIM (<i>n</i> = 1)	1	0	0	100	100.0	20.7	100.0	100.0	96.3	100.0	
		Overall (<i>n</i> = 85)	85	0	0	16	100.0	95.7	100.0	100.0	80.6	100.0	
	<i>P. aeruginosa</i> ^d (9)	VIM (<i>n</i> = 1)	1	0	0	8	100.0	20.7	100.0	100.0	67.6	100.0	
		IMP (<i>n</i> = 3)	3	0	0	6	100.0	43.9	100.0	100.0	61.0	100.0	
		Overall (<i>n</i> = 4)	4	0	0	5	100.0	51.0	100.0	100.0	56.6	100.0	
<i>Enterobacteriales</i> + <i>P. aeruginosa</i> (110)	Overall (<i>n</i> = 89)	89	0	0	21	100.0	95.9	100.0	100.0	84.5	100.0		
MacConkey agar	<i>Enterobacteriales</i> (101)	KPC (<i>n</i> = 26)	26	0	0	75	100.0	87.1	100.0	100.0	95.1	100.0	
		OXA-48-like (<i>n</i> = 15)	15	0	0	86	100.0	79.6	100.0	100.0	95.7	100.0	
		VIM (<i>n</i> = 7)	7	0	0	94	100.0	64.6	100.0	100.0	96.1	100.0	
		IMP (<i>n</i> = 6)	6	1 ^b	0	94	100.0	61.0	100.0	98.9	94.3	99.8	
		NDM (<i>n</i> = 27)	27	0	0	74	100.0	87.5	100.0	100.0	95.1	100.0	
		OXA-48 + NDM (<i>n</i> = 3)	3	0	0	98	100.0	43.9	100.0	100.0	96.2	100.0	
		OXA-48 + VIM (<i>n</i> = 1)	1	0	0	100	100.0	20.7	100.0	100.0	96.3	100.0	
		Overall (<i>n</i> = 85)	85	1	0	15	100.0	95.7	100.0	93.8	71.7	98.9	
	<i>Enterobacteriales</i> + <i>P. aeruginosa</i> (110)	Overall (<i>n</i> = 89)	89	1	0	20	100.0	95.9	100.0	95.2	77.3	99.2	
	Mueller-Hinton agar	<i>Enterobacteriales</i> (101)	KPC (<i>n</i> = 26)	26	0	0	75	100.0	87.1	100.0	100.0	95.1	100.0
			OXA-48-like (<i>n</i> = 15)	15	0	0	86	100.0	79.6	100.0	100.0	95.7	100.0
VIM (<i>n</i> = 7)			7	0	0	94	100.0	64.6	100.0	100.0	96.1	100.0	
IMP (<i>n</i> = 6)			5	0	1 ^c	95	83.3	43.7	97.0	100.0	96.1	100.0	
NDM (<i>n</i> = 27)			27	0	0	74	100.0	87.5	100.0	100.0	95.1	100.0	
OXA-48 + NDM (<i>n</i> = 3)			3	0	0	98	100.0	43.9	100.0	100.0	96.2	100.0	
OXA-48 + VIM (<i>n</i> = 1)			1	0	0	100	100.0	20.7	100.0	100.0	96.3	100.0	
Overall (<i>n</i> = 85)			84	0	1	16	98.8	93.6	99.8	100.0	80.6	100.0	
<i>Enterobacteriales</i> + <i>P. aeruginosa</i> (110)	Overall (<i>n</i> = 89)	88	0	1	21	98.9	93.9	99.8	100.0	84.5	100.0		

^aThese data are for 50 isolates from the Washington University isolate collection and 60 isolates from the challenge panel provided by Hardy Diagnostics. Testing was performed at site 4. TP, true positive; FP, false positive; TN, true negative; FN, false negative; 95% CI, 95% confidence interval.

^bOne *P. mirabilis* isolate was mCIM negative, IMP positive by NG-Test Carba 5, and characterized to harbor *bla*_{IMP-27} by whole-genome sequencing.

^cOne *P. rettgeri* isolate was mCIM positive, IMP negative by NG-Test Carba 5, and characterized to harbor *bla*_{IMP-27} by whole-genome sequencing.

^dThe performance with *P. aeruginosa* was the same for each agar type, so only data for blood agar are shown.

negative for IMP by NG-Test Carba 5 from Mueller-Hinton agar (positive mCIM result, negative NG-Test Carba 5 result, molecularly characterized to harbor *bla*_{IMP-27}). One *Proteus mirabilis* isolate, also from the Washington University strain bank, was considered false positive for IMP by NG-Test Carba 5 from MacConkey agar (negative mCIM result, positive NG-Test Carba 5 result, molecularly characterized to harbor *bla*_{IMP-27}). For *P. aeruginosa*, the PPA was 100% (95% CI, 51% to 100%) and the NPA was 100% (95% CI, 56.6% to 100%) for all agar types.

DISCUSSION

CP-CROs may be harbored in the human gastrointestinal tract and can be acquired in hospital settings or through food and travel (27, 28). While colonization may not always escalate into infection, the importance of identifying patients colonized and/or infected with CP-CROs has increased, since carbapenemase genes can easily spread between Gram-negative organisms in hospitals and in the community via plasmids (27, 28). Furthermore, detection of a nonendemic or rarely encountered carbapenemase can be important for infection control practices. On the therapeutic front, the importance of rapid carbapenemase differentiation immediately after carbapenem resistance detection is critical for treatment, as many novel compounds targeted against CP-CROs have specific activity

depending on the carbapenemase type (e.g., novel β -lactam- β -lactamase inhibitor combinations have no activity against MBLs, and certain agents, but not all of them, have activity against particular serine carbapenemases) (29, 30).

Overall, the multicenter clinical trial found that the PPA and the NPA for NG-Test Carba 5 compared to the composite reference method after discrepant analysis were 100%, regardless of the medium type. In this study, carbapenemase-producing *Enterobacterales* isolates were obtained from diverse body sites and source locations. In the future, with some enhancements, this test may be further validated to detect carbapenemases directly from different specimen types, hence augmenting the range of clinical applications (31, 32). Although all *P. aeruginosa* isolates prospectively enrolled at the clinical sites were negative by NG-Test Carba 5, 86.5% (32/37) of the *P. aeruginosa* isolates exhibited carbapenem resistance but were mCIM negative. These isolates may have mutations in the OprD porin combined with the hyperexpression of AmpC and/or efflux pumps (33). In these instances, the resistance mechanism is less likely to be plasmid mediated, but the resistance pattern remains a concern. Although carbapenemase production is a small contributor to carbapenem resistance among *P. aeruginosa* isolates in the United States (~2% of carbapenem-resistant *P. aeruginosa* isolates produce carbapenemases), there have been increasing reports of VIM-producing *P. aeruginosa* isolates in long-term care facilities and import to areas of nonendemicity due to medical tourism in areas of endemicity (34–38). Thus, it is important for clinical laboratories to have diagnostic tools available for carbapenemase detection in the event of changing epidemiology or an outbreak in the hospital setting.

The mCIM and eCIM test procedures showed excellent diagnostic performance characteristics, further supporting their use for broadly detecting carbapenemase activity. In this study, the majority of organisms positive by the NG-Test Carba 5 assay exhibited an mCIM zone diameter of 6 mm (i.e., growth of the *E. coli* ATCC 25922 reporter strain up to the disk), which confirmed the ease of interpretation of the mCIM test. The major drawback of the mCIM for clinical laboratories is the requirement of an overnight incubation step. This is a risk due to the high mortality rates associated with these infections or the potential for transmission in the hospital setting (5, 9–11). However, with the implementation of the NG-Test Carba 5 assay, the time to an actionable result is reduced to ~15 min for a phenotypic result for the five most common carbapenemase families.

A similar lateral flow assay, Resist-4 O.K.N.V., developed by Coris BioConcept (Gembloux, Belgium) (which detects KPC, NDM, OXA-48, and VIM enzymes), is yet to be cleared by the U.S. FDA but has shown good performance for all carbapenemases except NDM enzymes (39). An additional limitation of this assay is that it does not detect IMP enzymes and requires two devices for detecting the major carbapenemase families. Other commercially available phenotypic carbapenemase detection assays include the Rapidec CarbaNP assay (bioMérieux, Marcy-l'Étoile, France), while commercially available genotypic assays include the FilmArray blood culture identification panel (BioFire Diagnostics, LLC, Salt Lake City, UT), the Verigene Eplex assay (Luminex Corporation, Austin, TX), the BD Max CPO Detect assay (Becton, Dickinson and Company, Franklin Lakes, NJ), and the Xpert Carba-R assay, among others. To our knowledge, NG-Test Carba 5 is the most streamlined assay among the nongenotypic methods and offers differentiation among the major carbapenemase families. While direct-from-specimen platforms display excellent diagnostic characteristics, they require an upfront capital investment, which may not be possible for all laboratories. For laboratories that lack an efficient assay for carbapenemase detection and differentiation or institutions that look to simplify testing and reduce the use of molecular assays due to budgeting constraints, NG-Test Carba 5 can be an effective option to streamline the work flow and potentially reduce cost without affecting the overall quality of results.

Ultimately, these findings demonstrate the excellent performance of NG-Test Carba 5 for detecting and differentiating carbapenemase-producing *Enterobacterales* and *P. aeruginosa* isolates. The results of other single-center evaluations of NG-Test Carba 5 in France and the United Kingdom have been published and showed performance data

similar to those observed in our multicenter study, the first multicenter study to be described (40, 41). This was also the first study, to the authors' knowledge, to thoroughly evaluate the performance of NG-Test Carba 5 with colonies recovered from MacConkey agar, an important culture medium for the cultivation of Gram-negative bacteria. Furthermore, this study implemented blinding techniques and a standardized inoculation method with NG-Test Carba 5. These aspects of the study allowed for unbiased interpretation of the results when examining the NG-Test Carba 5 results and avoided extreme variations in sampling techniques across operators.

The inclusion of selective pressure on each agar medium is considered a limitation of the study, as most labs do not routinely place antimicrobial disks. *P. aeruginosa* isolates are intrinsically resistant to ertapenem; thus, no selective pressure was considered to have been applied for this species (19).

It has been well described that carbapenemase detection and differentiation are no longer solely for infection control or epidemiological purposes but are also of utmost importance for successful outcomes in patient care and antimicrobial stewardship due to the availability of novel antimicrobial agents that target specific carbapenemases (39–43). An area of future research will focus on the performance of NG-Test Carba 5 when applied directly to different specimen types, such as positive blood culture broths and urine. If successful, this will widen the clinical applicability of the NG-Test Carba 5 system.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

ACKNOWLEDGMENTS

This study was funded by Hardy Diagnostics and NG Biotech. All supplies (NG-Test Carba 5 devices, culture media, and all necessary laboratory supplies) were provided by Hardy Diagnostics and NG Biotech.

We thank Eric Wenzler for sharing strains for inclusion in the clinical study, and also Brian Johnson and Darcie Carpenter from International Health Management Associates, Inc. (IHMA), for conducting targeted PCR and bidirectional sequencing.

A.S.K., R.M., and A.H. are full-time employees and shareholders of Hardy Diagnostics and contributed to the study design, logistical coordination, data logging and analysis, and first draft of the manuscript. S.J., N.A.L., L.F.W., C.-A.D.B., and P.J.S. received research funds from Hardy Diagnostics to complete the study.

REFERENCES

- Pierce VM, Simner PJ, Lonsway DR, Roe-Carpenter DE, Johnson JK, Brasso WB, Bobenchik AM, Lockett ZC, Charnot-Katsikas A, Ferraro MJ, Thomson RB, Jenkins SG, Limbago BM, Das S. 2017. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacteriaceae. *J Clin Microbiol* 55:2321–2333. <https://doi.org/10.1128/JCM.00193-17>.
- Centers for Disease Control and Prevention. 2019. Antibiotic resistance threats in the United States, 2019. Centers for Disease Control and Prevention, Atlanta, GA. <https://www.cdc.gov/drugresistance/biggest-threats.html>.
- Centers for Disease Control and Prevention. 2013. Vital signs: carbapenem-resistant Enterobacteriaceae. *MMWR Morb Mortal Wkly Rep* 62:165–170.
- van Duin D, Kaye KS, Neuner EA, Bonomo RA. 2013. Carbapenem resistant Enterobacteriaceae: a review of treatment and outcomes. *Diagn Microbiol Infect Dis* 75:115–120. <https://doi.org/10.1016/j.diagmicrobio.2012.11.009>.
- Tamma PD, Goodman KE, Harris AD, Tekle T, Roberts A, Taiwo A, Simner PJ. 2017. Comparing the outcomes of patients with carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae bacteremia. *Clin Infect Dis* 64:257–264. <https://doi.org/10.1093/cid/ciw741>.
- Poiriel L, Pitout JD, Nordmann P. 2007. Carbapenemases: molecular diversity and clinical consequences. *Future Microbiol* 2:501–512. <https://doi.org/10.2217/17460913.2.5.501>.
- Doi Y, Paterson DL. 2015. Carbapenemase-producing Enterobacteriaceae. *Semin Respir Crit Care Med* 36:74–84. <https://doi.org/10.1055/s-0035-1544208>.
- Glasner C, Albiger B, Buist G, Tambić Andrašević A, Cantón R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Donker T, Monnet DL, Grundmann H, European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group. 2013. Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013. *Euro Surveill* 18(28):pii=20525. <https://doi.org/10.2807/1560-7917.ES2013.18.28.20525>.
- Guh AY, Limbago BM, Kallen AJ. 2014. Epidemiology and prevention of carbapenem-resistant Enterobacteriaceae in the United States. *Expert Rev Anti Infect Ther* 12:565–580. <https://doi.org/10.1586/14787210.2014.902306>.
- Gupta N, Limbago BM, Patel JB, Kallen AJ. 2011. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis* 53: 60–67. <https://doi.org/10.1093/cid/cir202>.
- Vasoo S, Barreto JN, Tosh PK. 2015. Emerging issues in gram-negative bacterial resistance: an update for the practicing clinician. *Mayo Clin Proc* 90:395–403. <https://doi.org/10.1016/j.mayocp.2014.12.002>.
- Tamma PD, Simner PJ. 2018. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. *J Clin Microbiol* 56:e01140-18. <https://doi.org/10.1128/JCM.01140-18>.
- van der Zee A, Roorda L, Bosman G, Fluit AC, Hermans M, Smits PHM, van

- der Zanden AGM, Te Witt R, Bruijnesteijn van Coppenraet LES, Stuart JC, Ossewaarde JM. 2014. Multi-centre evaluation of real-time multiplex PCR for detection of carbapenemase genes OXA-48, VIM, IMP, NDM and KPC. *BMC Infect Dis* 14:27. <https://doi.org/10.1186/1471-2334-14-27>.
14. Hardy Diagnostics. 2019. NG-Test CARBA 5 package insert. https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/NG-Test%20CARBA%205%20US_ENO019CAR_v191107.pdf.
 15. Zhou M, Kudinha T, Du B, Peng J, Ma X, Yang Y, Zhang G, Zhang J, Yang Q, Xu YC. 2019. Active surveillance of carbapenemase-producing organisms (CPO) colonization with Xpert Carba-R assay plus positive patient isolation proves to be effective in CPO containment. *Front Cell Infect Microbiol* 9:162. <https://doi.org/10.3389/fcimb.2019.00162>.
 16. U.S. Food and Drug Administration. 2016. Xpert Carba-R 510(k) substantial equivalence determination decision summary. https://www.accessdata.fda.gov/cdrh_docs/reviews/K152614.pdf.
 17. Tato M, Ruiz-Garbajosa P, Traczewski M, Dodgson A, McEwan A, Humphries R, Hindler J, Veltman J, Wang H, Cantón R. 2016. Multisite evaluation of Cepheid Xpert Carba-R assay for detection of carbapenemase-producing organisms in rectal swabs. *J Clin Microbiol* 54:1814–1819. <https://doi.org/10.1128/JCM.00341-16>.
 18. Cepheid. 2018. Xpert Carba-R package insert. [https://health.maryland.gov/laboratories/docs/Xpert%20Carba-R%20Assay-%203031-2438%20Rev%20B%20\(Package%20Insert\).pdf](https://health.maryland.gov/laboratories/docs/Xpert%20Carba-R%20Assay-%203031-2438%20Rev%20B%20(Package%20Insert).pdf).
 19. Clinical and Laboratory Standards Institute. 2020. Performance standards for antimicrobial susceptibility testing, vol M100. Approved standard, 30th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
 20. Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial disk susceptibility tests. CLSI standard M02, 13th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
 21. Sfeir MM, Hayden JA, Fautleroy KA, Mazur C, Johnson JK, Simner PJ, Das S, Satlin MJ, Jenkins SG, Westblade LF. 2019. EDTA-modified carbapenem inactivation method: a phenotypic method for detecting metallo- β -lactamase-producing *Enterobacteriaceae*. *J Clin Microbiol* 57:e01757-18. <https://doi.org/10.1128/JCM.01757-18>.
 22. Simner PJ, Johnson JK, Brasso WB, Anderson K, Lonsway DR, Pierce VM, Bobenchik AM, Lockett ZC, Charnot-Katsikas A, Westblade LF, Yoo BB, Jenkins SG, Limbago BM, Das S, Roe-Carpenter DE. 2017. Multicenter evaluation of the modified carbapenem inactivation method and the Carba NP for detection of carbapenemase-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *J Clin Microbiol* 56:e01369-17. <https://doi.org/10.1128/JCM.01369-17>.
 23. Kazmierczak KM, Lob SH, Hoban DJ, Hackel MA, Badal RE, Bouchillon SK. 2015. Characterization of extended-spectrum β -lactamases and antimicrobial resistance of *Klebsiella pneumoniae* in intra-abdominal infection isolates in Latin America, 2008–2012. Results of the study for monitoring antimicrobial resistance trends. *Diagn Microbiol Infect Dis* 82:209–214. <https://doi.org/10.1016/j.diagmicrobio.2015.03.025>.
 24. Nichols WW, de Jonge BLM, Kazmierczak KM, Karlowsky JA, Sahn DF. 2016. *In vitro* susceptibility of global surveillance isolates of *Pseudomonas aeruginosa* to ceftazidime-avibactam (INFORM 2012 to 2014). *Antimicrob Agents Chemother* 60:4743–4749. <https://doi.org/10.1128/AAC.00220-16>.
 25. Clinical and Laboratory Standards Institute. 2008. User protocol for evaluation of qualitative test performance; approved guideline, 2nd ed. CLSI document EP12-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
 26. Altman DG, Machin D, Bryant TN, Gardner MJ. 2000. *Statistics with confidence*, 2nd ed. BMJ Books, London, Great Britain.
 27. Bush K, Fisher JF. 2011. Epidemiological expansion, structural studies, and clinical challenges of new β -lactamases from gram-negative bacteria. *Annu Rev Microbiol* 65:455–478. <https://doi.org/10.1146/annurev-micro-090110-102911>.
 28. Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL, European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group. 2015. Carbapenemase-producing *Enterobacteriaceae* in Europe: assessment by national experts from 38 countries. *Euro Surveill* 20(25):pii=30062. <https://doi.org/10.2807/1560-7917.ES.2015.20.45.30062>.
 29. Stone GG, Bradford PA, Yates KJ, Newell P. 2017. *In vitro* activity of ceftazidime/avibactam against urinary isolates from patients in a phase 3 clinical trial programme for the treatment of complicated urinary tract infections. *J Antimicrob Chemother* 72:1396–1399.
 30. Pfizer. 2017. Pfizer Launches Zavicefta™ (ceftazidime-avibactam) in the U.K. and Germany, a new antibiotic to treat complicated infections caused by Gram-negative bacteria. <https://investors.pfizer.com/investor-news/press-release-details/2017/Pfizer-Launches-Zavicefta-ceftazidime-avibactam-in-the-UK-and-Germany-a-New-Antibiotic-to-Treat-Complicated-Infections-Caused-by-Gram-Negative-Bacteria/default.aspx>.
 31. Takissian J, Bonnin RA, Naas T, Dortet L. 2019. NG-test Carba 5 for rapid detection of carbapenemase-producing *Enterobacteriales* from positive blood cultures. *Antimicrob Agents Chemother* 25:e00011-19. <https://doi.org/10.1128/AAC.00011-19>.
 32. Bodendoerfer E, Keller PM, Mancini S. 2019. Rapid identification of NDM-, KPC-, IMP-, VIM- and OXA-48-like carbapenemase-producing *Enterobacteriales* from blood cultures by a multiplex lateral flow immunoassay. *J Antimicrob Chemother* 74:1749–1751. <https://doi.org/10.1093/jac/dkz056>.
 33. Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. 2012. Mechanisms responsible for the emergence of carbapenem resistance in *Pseudomonas aeruginosa*. *Hippokratia* 16:303–307.
 34. Woodworth KR, Walters MS, Weiner LM, Edwards J, Brown AC, Huang JY, Malik S, Slayton RB, Paul P, Capers C, Kainer MA, Wilde N, Shugart A, Mahon G, Kallen AJ, Patel J, McDonald LC, Srinivasan A, Craig M, Cardo DM. 2018. Vital signs: containment of novel multidrug-resistant organisms and resistance mechanisms—United States, 2006–2017. *MMWR Morb Mortal Wkly Rep* 67:396–401. <https://doi.org/10.15585/mmwr.mm6713e1>.
 35. Walters MS, Grass JE, Bulens SN, Hancock EB, Phipps EC, Muleta D, Mounsey J, Kainer MA, Concannon C, Dumyati G, Bower C, Jacob J, Cassidy PM, Beldavs Z, Culbreath K, Phillips WE, Hardy DJ, Vargas RL, Oethinger M, Ansari U, Stanton R, Albrecht V, Halpin AL, Karlsson M, Rasheed JK, Kallen A. 2019. Carbapenem-resistant *Pseudomonas aeruginosa* at US emerging infections program sites, 2015. *Emerg Infect Dis* 25:1281–1288. <https://doi.org/10.3201/eid2507.181200>.
 36. Clegg WJ, Pacilli M, Kemble SK, Kerins JL, Hassaballa A, Kallen AJ, Walters MS, Halpin AL, Stanton RA, Boyd S, Gable P, Daniels J, Lin M, Hayden MK, Lolans K, Burdsall DP, Lavin MA, Black SR. 2018. Notes from the field: large cluster of Verona integron-encoded metallo-beta-lactamase-producing carbapenem-resistant *Pseudomonas aeruginosa* isolates colonizing residents at a skilled nursing facility—Chicago, Illinois, November 2016–March 2018. *MMWR Morb Mortal Wkly Rep* 67:1130–1131. <https://doi.org/10.15585/mmwr.mm6740a6>.
 37. Centers for Disease Control and Prevention. 2019. Verona integron-encoded metallo-beta-lactamase-producing carbapenem-resistant *Pseudomonas aeruginosa* infections associated with elective invasive medical procedures in Mexico—multiple U.S. states, 2018–2019. https://www.cdc.gov/eis/conference/dpk/Resistant_Pseudomonas_Aeruginosa_Infections.html.
 38. Gutiérrez O, Juan C, Cercenado E, Navarro F, Bouza E, Coll P, Pérez JL, Oliver A. 2007. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from Spanish hospitals. *Antimicrob Agents Chemother* 51:4329–4335. <https://doi.org/10.1128/AAC.00810-07>.
 39. Kolenda C, Benoit R, Carricajo A, Bonnet R, Dauwalder O, Laurent F. 2018. Evaluation of the new multiplex immunochromatographic O.K.N.V. K-SeT assay for rapid detection of OXA-48-like, KPC, NDM, and VIM carbapenemases. *J Clin Microbiol* 56:e01247-18. <https://doi.org/10.1128/JCM.01247-18>.
 40. Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G, Plaisance M, Oueslati S, Dortet L, Jousset A, Simon S, Naas T, Volland H. 2018. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing *Enterobacteriaceae*. *J Antimicrob Chemother* 73:909–915. <https://doi.org/10.1093/jac/dkx521>.
 41. Hopkins KL, Meunier D, Naas T, Volland H, Woodford N. 2018. Evaluation of the NG-Test CARBA 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases. *J Antimicrob Chemother* 73:3523–3526. <https://doi.org/10.1093/jac/dky342>.
 42. Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, Westblade LF. 2017. Carbapenemase-producing organisms: a global scourge. *Clin Infect Dis* 66:1290–1297. <https://doi.org/10.1093/cid/cix893>.
 43. Banerjee R, Humphries R. 2017. Clinical and laboratory considerations for the rapid detection of carbapenem-resistant *Enterobacteriaceae*. *Virulence* 8:427–439. <https://doi.org/10.1080/21505594.2016.1185577>.