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Comparison of a Novel, Rapid Chromogenic Biochemical Assay, the Carba NP Test, with the Modified Hodge Test for Detection of Carbapenemase-Producing Gram-Negative Bacilli

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We compared carbapenemase detection among 271 Gram-negative bacilli (of which 131 were carbapenemase producers) using a novel chromogenic rapid test—the Carba NP test (CNP)—and the modified Hodge test (MHT). Sensitivities were comparable (CNP, 100%, versus MHT, 98%; $P = 0.08$), but CNP was more specific (100% versus 80%; $P < 0.0001$) and faster.

Gram-negative bacilli (GNB) with acquired carbapenemases have disseminated worldwide, rendering them a global threat. The therapeutic armamentarium for infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) is limited, and CRE infections have been associated with significant mortality (1). *Enterobacteriaceae* harboring *Klebsiella pneumoniae* carbapenemase (KPC) are now endemic in some regions of the United States (2, 3), and although still sporadic, GNB harboring New Delhi metallo-β-lactamase (NDM) have been reported for nine states (4). Timely detection of these plasmid-borne and easily transmissible carbapenemases (along with emerging carbapenemases like OXA-48 and VIM) is important given implications for appropriate therapy and infection control. Detection is challenging, since isolates may have only borderline reductions in susceptibility to carbapenems (5), and resistance may be mediated by mechanisms other than carbapenemase production (e.g., AmpC or extended-spectrum β-lactamase [ESBL] with decreased membrane permeability). While molecular methods are confirmatory, testing may not be immediately available and may be limited by the number of targets assayed. The modified Hodge test (MHT), a CLSI-recommended confirmatory test for carbapenemase production, suffers from lack of specificity, a long turnaround time, and poor sensitivity for metallo-β-lactamase detection (6, 7). A rapid phenotypic test to screen for carbapenemases is highly desirable. Recently, Nordmann et al. described a rapid chromogenic carbapenemase detection assay based on hydrolysis of the β-lactam ring of imipenem, the Carba NP test (CNP) (8–10). Herein, we compared the CNP to the MHT for detection of carbapenemase-producing GNB.

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Two hundred seventy-one GNB, including 39 characterized reference isolates (5 derepressed AmpC mutants and 5 plasmid-mediated AmpC producers with or without porin loss, 18 ESBL producers with or without porin loss, and 8 KPC, 1 NDM-1, 1 OXA-48, and 1 VIM-2 isolate) and 232 clinical isolates (including 111 KPC, 3 NDM, 1 SME-1, and 5 VIM isolates), were studied (Table 1). The 271 isolates included 201 *Enterobacteriaceae* and 70 nonfermenting GNB. Clinical isolates included those submitted to the clinical microbiology laboratory, Mayo Clinic, Rochester, MN, from October 2012 to January 2013 for modified Hodge testing ($n = 40$) and rectal swab surveillance isolates ($n = 192$). The latter were recovered using the Centers for Disease Control and Prevention (CDC)-recommended method for CRE screening (11) and/or on HardyCHROM ESBL medium (Hardy Diagnostics, Santa Maria, CA) from 47 rectal surveillance swabs collected between September and November 2012, as part of a CRE colonization study performed in long-term acute-care facilities in Chicago, IL (12). The MHT was performed using Mueller-Hinton Agar (BD BBL, Franklin Lakes, NJ) with 10 μg meropenem and ertapenem disks (BD BBL). The CNP, with slight modification from the originally described protocol (modifications based on personal communication from Patrice Nordmann), was performed as follows. Each isolate was tested in paired tubes. Two 1.5-ml low-bind protein microcentrifuge tubes (Eppendorf NA, Hauppauge, NY), each containing 100 μl of a 20 mM tris-HCl lysis buffer, SoluLyse (Thermo Fisher Scientific, Waltham, MA), were individually inoculated with a 1-μl loopful of bacterial colony (18 to 24 h old, loop swept through pure culture), and bacterial suspensions were vortexed for 5 s. To the first tube, 100 μl of 0.5% (wt/vol) phenol red solution (Sigma-Aldrich, St. Louis, MO) with 10 mM zinc sulfate (Sigma-Aldrich) (solution A, buffered to pH 7.8 by adding 0.1 N NaOH) was then added, and the tube was vortexed. To the second tube, 100 μl of solution A with imipenem (USP) dissolved directly in solution A to a final concentration of 6 mg/ml was added and then vortexed. Imipenem was reconstituted in solution A on each day of testing. *K. pneumoniae* ATCC BAA1705 (KPC positive) and BAA1706 (KPC negative) and a pair of "blank" tubes without bacteria were used as controls with each CNP run. Tubes were visually read at 15, 30, 45, 60, and 120 min. A color change from red to yellow/orange indicated carbapenemase production (i.e., as a result of the pH change induced by

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TABLE 1 Breakdown of reference and clinical isolates tested

Description	Species (n^o)	Genotype(s) (n^o)	MIC of drug		Test result(s) (n^p)	
			Ertapenem	Meropenem	Carba NP	Modified Hodge
Reference isolates						
Derepressed AmpC mutant[a]	*E. coli* (3)	*ampC* promoter/attenuator mutation (3)	≤0.25	≤1	Negative (3)	Negative (2), positive (1)[d]
Derepressed AmpC mutant with porin loss[a]	*E. coli* (1)	*ampC* attenuator mutation and OmpF	1	≤1	Negative (1)	Equivocal[e]
	E. coli (1)	*ampC* promoter/attenuator mutation and OmpC	≤0.25	≤1	Negative (1)	Positive[d]
AmpC[a]	*E. coli* (3)	CMY-2	≤0.25–0.5	≤1	Negative (3)	Negative (2), positive (1)[d]
	E. coli (1)	FOX-5	≤0.25	≤1	Negative	Equivocal[d]
AmpC with porin loss[a]	*E. coli* (1)	CMY-2 and OmpF	≤0.25	≤1	Negative	Equivocal[e]
ESBL[b]	*E. coli* (1)	TEM-1 + SHV-43	≤0.25	≤1	Negative	Negative
	K. pneumoniae (6)	CTX-M-12, SHV-43, TEM-10, TEM-10 + TEM-1, SHV-12, TEM-9	≤0.25	≤1	Negative (6)	Negative (6)
ESBL with porin loss	*E. coli* (7)	CTX-M-15, TEM-1 + OmpF[a] (2); CTX-M-15, OXA-1 + OmpC[a] (2); CTX-M-14, TEM-1 + OmpF[a] (2), TEM-12 + OmpC/F[b] (1)	≤0.25–1	≤1	Negative (7)	Negative (6), equivocal (1)[e]
	K. pneumoniae (4)[a]	CTX-M-2, SHV-11 + OmpK35 (1); CTX-M-15, SHV-11, TEM-1, OXA-1 + OmpK36 (1); CTX-M-15, OXA-1 + OmpK35/36 (1); CTX-M-15, SHV-1 + OmpK35 (1)	≤0.25–0.5	≤1	Negative (4)	Negative (4)
Acquired carbapenemases	*Citrobacter* spp. (1)	KPC	>4	8	Positive	Positive
	E. aerogenes (1)	KPC	>4	>8	Positive	Positive
	E. cloacae complex (1)	KPC	>4	8	Positive	Positive
	E. coli (1)	KPC	4	2	Positive	Positive
	P. aeruginosa (1)	KPC	>4	>8	Positive	Positive
	P. mirabilis (1)	KPC	>4	8	Positive	Positive
	P. stuartii (1)	KPC	≤0.25	≤1	Positive	Positive
	S. marcescens (1)	KPC	>4	>8	Positive	Positive
	E. coli (1)[c]	NDM-1	>4	>8	Positive	Positive
	K. pneumoniae NCTC 13442 (1)	OXA-48	1	≤1	Positive	Positive
	P. aeruginosa (1)[b]	VIM-2	>4	>8	Positive	Positive
Clinical isolates						
Carbapenem-resistant *Enterobacteriaceae* (carbapenemase producing)	*K. pneumoniae* (98)	KPC	>4/>4	8/>8	Positive (111)	Positive (111)
	Klebsiella spp. (3)	KPC				
	E. coli (4)	KPC				
	Citrobacter spp. (2)	KPC				
	E. cloacae complex (1)	KPC				
	E. aerogenes (1)	KPC				
	P. stuartii (1)	KPC				
	S. marcescens (1)	KPC				
	K. pneumoniae (2), *E. coli* (1)	NDM	>4/>4	>8/>8	Positive (3)	Positive (1), Negative (2)
	S. marcescens (1)	SME-1	4	8	Positive	Positive
Carbapenem-resistant *Enterobacteriaceae* (non-carbapenemase producing)	*E. aerogenes* (1)	*ampC* + *ompK36*	>4	4	Negative	Negative
	E. cloacae complex (2)	Undefined (ESBL phenotype; likely hyperproducer) (1); *ampC* (1)	1	<1	Negative	Positive[f]
	E. coli (6)	*ampC* (4); Undefined (ESBL phenotype; likely hyperproducer) (2)	>4/>4	4/>8	Negative (6)	Negative (4), positive (1)[g], inhibited (1)[h]
	K. pneumoniae (2)	*ompK35/36* (1); *ampC*+*ompK35* (1)	2–>4	≤1–2	Negative (2)	Negative (2)
	P. agglomerans (1)	*ampC*	>4	4	Negative	Negative
Enterobacteriaceae resistant to expanded-spectrum cephalosporins	*E. coli* (4)	Not done	≤0.25/≤0.25	≤1/≤1	Negative (17)	Negative (15), positive (2)[i]
	K. pneumoniae (4)	Not done				
	P. mirabilis (3)	Not done				
	P. stuartii (3)	Not done				
	M. morganii (2)	Not done				
	E. cloacae complex (1)	Not done				
Expanded-spectrum cephalosporin/carbapenem-susceptible *Enterobacteriaceae*	*P. stuartii* (4)	Not done	≤0.25/≤0.25	≤1/≤1	Negative (20)	Negative (16), positive (2)[j], inhibited (2)[k]
	P. mirabilis (5)	Not done				
	E. coli (5)	Not done				
	K. pneumoniae (2)	Not done				
	E. aerogenes (2)	Not done				
	M. morganii (1)	Not done				
	Serratia liquefaciens/*S. grimesii* (1)	Not done				

Carbapenem-resistant nonfermenting Gram-negative bacilli	*P. aeruginosa* (51)	VIM (5)[i]; not done (62)	>4/>4	>8/>8	Positive(5)[j] Negative (62)	Positive (18)[m] Negative (36), inhibited (13)[n]
	Acinetobacter spp. (16)		>8/>8	>8/>8		
Carbapenem-susceptible nonfermenting Gram-negative bacilli	*Alcaligenes faecalis* (1)	Not done (1)	≤0.25	≤1	Negative	Negative

[a] Gift of George Zhanel and Daryl Hoban.
[b] Gift of John Quinn.
[c] Gift of Paul Schreckenberger.
[d] Ertapenem disk.
[e] Meropenem disk.
[f] Ertapenem disk Modified Hodge Test (MHT) positive, meropenem disk MHT weak positive.
[g] One isolate with *ampC*.
[h] One isolate with *ampC*.
[i] One *Proteus mirabilis* and one *Providencia stuartii* isolate.
[j] Two *P. stuartii* isolates.
[k] Two *P. mirabilis* isolates.
[l] Conventional PCR was done for five *Pseudomonas aeruginosa* isolates positive for the Carba NP test, and these were found to be positive for VIM.
[m] Six *P. aeruginosa* and 12 *Acinetobacter* isolates.
[n] Twelve *P. aeruginosa* isolates and one *Acinetobacter* isolate.
[o] *n*, no. of isolates.
[p] *n*, no. of results.

FIG 1 Carba NP test: example of assay and interpretation of results. (a) KPC *Providencia stuartii* (ertapenem MIC ≤ 0.25 μg/ml; meropenem MIC ≤ 1 μg/ml) Carba NP test run; note relative slower color changes. The majority of KPC-positive isolates in this study turned a definitive yellow within 15 min. (b) Carba NP read at 2 h. Isolates were tested in paired tubes; those labeled "a" contain solution A, and tubes labeled "b" contain solution A plus imipenem. Tubes 1, KPC-positive *Klebsiella pneumoniae* control (BAA-1705); tubes 2, KPC *K. pneumoniae*-negative control (BAA-1706); tubes 3, blank control; tubes 4, extended-spectrum-beta-lactamase-producing *Escherichia coli* (TEM-12); tubes 5, NDM-1-positive *E. coli*; tubes 6, OXA-48-positive *K. pneumoniae* (NCTC 13442).

imipenem hydrolysis), while tubes remaining red/reddish-orange were considered negative (Fig. 1). Clinical isolates were tested in a blinded fashion. Interobserver accuracy was assessed by three blinded operators who performed the CNP in triplicate using four isolates (1 NDM *Escherichia coli* isolate, 1 VIM-2 *Pseudomonas aeruginosa* isolate, 1 KPC isolate [ATCC BAA-1705], and one non-carbapenemase-producing isolate [ATCC BAA-1706]) on different days. A duplex PCR for bla_{KPC} and bla_{NDM} was performed with clinical/surveillance isolates (13). If negative for bla_{KPC} and bla_{NDM}, CRE were further evaluated for SME (14), VIM (15), IMP (16), GES (17), and OXA-48 (18), AmpC (19), and

TABLE 2 Performance characteristics of the Carba NP and modified Hodge tests

Performance characteristic (compared to presence of carbapenemase gene)	Value for isolate group and test (n^e)[a]			
	All isolates (*Enterobacteriaceae* and nonfermenting Gram-negative bacilli)		*Enterobacteriaceae*	
	Carba NP (269)[b]	Modified Hodge (251)[b]	Carba NP (200)[c]	Modified Hodge (194)[c]
Sensitivity, % (95% CI[d])	100 (96.4–100)	97.7 (92.9–99.4)	100 (96.3–100)	98.4 (93.7–99.7)
Specificity, % (95% CI)	100 (96.7–100)	80.0 (71.5–86.5)	100 (94.1–100)	85.7 (74.8–92.6)
Positive predictive value, % (95% CI)	100 (96.4–100)	84.2 (77.2–89.4)	100 (96.3–100)	92.4 (86.1–96.1)
Negative predictive value, % (95% CI)	100 (96.7–100)	97.0 (90.8–99.2)	100 (94.1–100)	96.8 (87.8–99.4)

[a] Excluding equivocal/nonreadable results for the MHT.
[b] McNemar's test for sensitivity, $P = 0.08$; specificity, $P < 0.0001$.
[c] McNemar's test for sensitivity, $P = 0.16$; specificity, $P < 0.0001$.
[d] CI, confidence interval.
[e] *n*, no. of isolates.

OmpK35/36 porin loss (20) using conventional PCR. Nonfermenting GNB which yielded a positive CNP result were also subjected to the aforementioned conventional PCRs. PCR was considered the gold standard for carbapenemase characterization. Antimicrobial susceptibility testing was performed by agar dilution, and identification of clinical isolates was by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Billerica, MA).

The CNP detected all carbapenemase-producing positive GNB ($n = 131$), while the MHT detected 128/131 (97.7%). Two NDM isolates were negative by the MHT. The two tests had comparable overall sensitivities ($P = 0.08$), but the CNP had superior specificity overall and with the subset of *Enterobacteriaceae* (both $P < 0.0001$) (Table 2). Besides inferior specificity, the MHT yielded 20 (7.4%) equivocal/nonreadable results (i.e., growth inhibition of the *Escherichia coli* lawn around the test streak or swarming of *Proteus* species). All positive CNP reactions yielded a distinct color change to yellow on initial testing except for two VIM-positive *Pseudomonas aeruginosa* isolates (one reference and one clinical isolate), which yielded orange and light orange colors, respectively. These reactions were considered positive but were repeated by a second operator for confirmation. Both isolates yielded a yellow reaction on repeat testing. Overall, of the CNP-positive isolates ($n = 131$), 123/131 (93.9%) and 129/131 (98.5%) turned distinctly yellow (positive) at 15 and 60 min, respectively. Eight isolates took more than 15 min to achieve a distinct yellow reaction: these comprised 5 VIM *P. aeruginosa* (range, 30 min to 2 h), 1 OXA-48 *Klebsiella pneumoniae* (1 h), 1 KPC *K. pneumoniae* (30 min), and 1 *Providencia stuartii* (45 min) isolate. There was 100% accuracy and agreement in the interobserver CNP assessment.

The CDC and Public Health England (formerly the Health Protection Agency) recommended active surveillance for CRE in certain high-risk settings or for certain patient groups (e.g., patients transferred from long-term-care facilities) (21, 22). Although the CDC recommends testing CRE isolates from patients with a history of hospitalization outside the United States for bla_{KPC} and bla_{NDM} (23), molecular testing is largely limited to public health and reference laboratories. The MHT, while a useful screen for carbapenemases, suffers from lack of specificity, poor sensitivity for metallo-β-lactamase detection, and a long turnaround time, findings corroborated by our study. Although we studied a limited number of NDM isolates, the CNP detected all four, compared to two of four detected by the MHT. This is likely due to zinc supplementation of the CNP (7). While the CNP detected all carbapenemase-producing GNB in our study, we noticed that color changes were slower for VIM-positive isolates and isolates with low carbapenem MICs (the KPC *P. stuartii* and OXA-48 *K. pneumoniae* reference isolates), possibly secondary to inherent differences in hydrolytic activities of VIM and OXA-48 versus KPC and NDM carbapenemases (24–26) and/or lower expression of carbapenemases in the KPC *P. stuartii* isolate with low carbapenem MICs (27). The blank tubes included in each run were helpful in this respect; true-positive isolates should exhibit a yellow/orange color, which is more obvious in comparison to the blank. A limitation of our study is that the majority of carbapenemase-producing *Enterobacteriaceae* studied were KPC-positive isolates; specificity would have been more rigorously assessed had we studied more carbapenemase-negative, carbapenem-nonsusceptible *Enterobacteriaceae*. However, a strength of our study was that the CNP was assessed with clinical isolates cultured from surveillance swabs and not just on reference isolates alone, thus decreasing selection bias.

Our findings are consistent with previously published data by Nordmann and colleagues (8–10), although in this study we utilized whole bacterial cells (instead of supernatant after bacterial lysis) and an increased concentration of imipenem (6 instead of 3 mg/ml) based on personal communication with Nordmann. This approach enabled detection of a KPC *P. stuartii* isolate, 6 VIM *P. aeruginosa* isolates (1 reference, and 5 clinical), and 1 KPC *P. aeruginosa* isolate which were initially negative when tested with 3 mg/ml of imipenem and supernatant obtained after bacterial lysis (8; also data not shown). Overall, we found that the CNP was easily performed, displayed excellent sensitivity, and had superior specificity and a faster turnaround time than the MHT. These features allow easy implementation in the clinical laboratory and translate to timely and actionable clinical results.

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