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Evaluation of the Carba NP Test for Rapid Detection of Carbapenemase-Producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*

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The Carba NP test was evaluated against a panel of 244 carbapenemase- and non-carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates. We confirmed the 100% specificity and positive predictive value of the test, but the sensitivity and negative predictive value were 72.5% and 69.2%, respectively, and increased to 80% and 77.3%, respectively, using a more concentrated bacterial extract. False-negative results were associated with mucoid strains or linked to enzymes with low carbapenemase activity, particularly OXA-48-like, which has emerged globally in enterobacteria.

The Carba NP test is a novel phenotypic method developed for carbapenemase detection (1, 2). It is based on *in vitro* hydrolysis of imipenem by a bacterial lysate, which is detected by changes in pH values using the indicator phenol red (red to yellow/orange). It was reported to be 100% sensitive and specific for *Enterobacteriaceae* and 100% specific and 94.4% sensitive for *Pseudomonas* spp. harboring carbapenemases (1, 2). The goal of this study was to evaluate this test using carbapenemase and non-carbapenemase producers of various Gram-negative species.

(Part of this research was presented as posters at the 2013 AMMI Canada-CACMID Annual Conference and the 113rd General Meeting of the American Society for Microbiology [3, 4].)

A panel of 244 *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates was tested: 145 containing different class of carbapenemases (KPC, NDM, VIM, IMP, OXA-48-like, IMI/NMC, and SME), all confirmed by PCR and sequence analysis, and 99 non-carbapenemase producers (Table 1), 57 of which were resistant to at least one of the carbapenems tested. In the latter group, phenotypic tests for carbapenemase production (modified Hodge test and KPC/MBL Confirm Kit; Rosco Diagnostica) were also negative, which led us to conclude that the carbapenem resistance observed was due to overexpression of chromosomal AmpC or expression of plasmid-mediated AmpC and/or extended-spectrum β-lactamases (ESBLs) coupled to impermeability. Carbapenem MICs were determined by Etest or agar dilution, and the results were interpreted using the Clinical and Laboratory Standards Institute guidelines (5). The Carba NP test was performed on strains grown on Mueller-Hinton agar plates as previously described (1, 2) in triplicate for each isolate, and results were interpreted by more than one independent reader. In the cases where false-negative results were obtained, two modifications of the procedure were attempted. First, 0.1-mm zirconia beads (ratio, 1:1 [vol/vol]) were added to the bacterial suspension before vortexing to improve lysis. Second, more concentrated extracts were made by increasing the amount of bacteria used to three to four calibrated 10-μl loopfuls in 200 μl of lysis buffer. To validate the results obtained and the protocol's modifications, blind samples of 74 isolates (37 from each laboratory) were interchanged between two independent laboratories; samples included carbapenemase producers ($n = 25$; 5 IMP, 4 KPC, 5 NDM, 2 VIM, 6 OXA-48-like, and 2 SME producers and 1 NMC/IMI producer), negative controls ($n = 13$), and strains with false-negative results ($n = 36$; 1 NMC-A, 4 GES-5, 4 SME-1, 24 OXA-48-like, 1 IMP-27, and 2 NDM-1 producers).

Using the original protocol (1, 2), all non-carbapenemase producers were negative by the Carba NP test (Table 1), including some strains with high carbapenem MICs (≥32 μg/ml), which confirmed the specificity and positive predictive value (100%) of the method described previously (1, 2). All KPC and VIM producers and most isolates producing NDM (31/33), IMP (6/7), and NMC/IMI (6/7) were detected by the test. On the other hand, after several attempts, the test failed to detect one *Proteus mirabilis* isolate expressing IMP-27, one NMC-A-producing *Enterobacter cloacae* isolate, and NDM-1-producing *Providencia rettgeri* ($n = 1$) and *Providencia stuartii* ($n = 1$) (Table 1). False-negative results were also obtained on GES-5 producers (5/5), SME-1-producing *Serratia marcescens* (4/8), and OXA-48-like producers (31/39). These results reduced the sensitivity and negative predictive value (NPV) of the test to 72.5% and 69.2%, respectively. False-negative results were associated with strains presenting mucoid colonies (e.g., NCM-A-producing *E. cloacae*, NDM-producing *P. rettgeri*, and some OXA-48-producing *Klebsiella pneumoniae* isolates) or linked to enzymes with weak carbapenemase activity (e.g., OXA-48-like or GES-5) although reduced carbapenemase expression in these isolates could not be excluded. The negative Carba NP results in *P. aeruginosa* isolates harboring GES-5 were consistent with what was originally demonstrated (2). An *Escherichia coli* J53 transconjugant containing the NDM plasmid of *P. rettgeri* and *E. coli* TOP10 (Life Technologies) transformed with a cloned bla_{IMP-27} gene from *P. mirabilis* were positive for the test, suggesting incomplete lysis or low carbapenemase gene expression in at least some clinical isolates. To rule out poor lysis as a factor in

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TABLE 1 Results of the Carba NP test

Species (*n*)[a]	Carbapenemase detected (*n*)[a]	MIC(s) (μg/ml)			Test result[b]
		Imipenem	Meropenem	Ertapenem	
Non-carbapenemase producers[c]					
Citrobacter freundii (3)	None	2–3	0.5–3	1.5–12	−
Enterobacter aerogenes (4)	None	0.5–4	0.06–1.5	0.12–16	−
Enterobacter cloacae (28)	None	0.19–6	0.06 to ≥32	0.25	−
Escherichia coli (20)	None	0.023–3	0.023–12	0.008 to ≥32	−
Klebsiella oxytoca (1)	None	0.25	0.25	1	−
Klebsiella pneumoniae (18)	None	0.19–4	0.016–12	0.016 to ≥32	−
Morganella morganii (1)	None	2	0.12	0.25	−
Providencia stuartii (1)	None	1.5	0.12	0.5	−
Pantoea spp. (1)	None	0.38	0.047	0.19	−
Serratia fonticola (1)	None		0.38	4	−
Serratia marcescens (6)	None	0.38–4	0.023–0.5	0.25–2	−
Pseudomonas aeruginosa (15)[d]	None	6 to ≥32	3 to ≥32	ND	−
Carba NP test-positive carbapenemase producers[e]					
Citrobacter freundii (3)	KPC (2)	6–12	2–6	6–4	+
	NDM (1)	4	1.5	6	+
Citrobacter youngae (1)	KPC	16	6	≥32	+
Enterobacter cloacae (25)	KPC (11)	0.75 to ≥32	0.38 to ≥32	0.75 to ≥32	+
	NDM (4)	2 to ≥32	0.75 to ≥32	2 to ≥32	+
	NMC/IMI (6)	≥32	6 to ≥32	16 to ≥32	+
	VIM (4)	6–24	6–32	2–24	+
Escherichia coli (33)	KPC (5)	1–16	0.25 to ≥32	0.5 to ≥32	+
	NDM (10)	4 to ≥32	2 to ≥32	12 to ≥32	+
	OXA-48 (14)	0.5–8	0.25–2	0.5 to ≥32	+
	VIM (3)	2–12	0.5–24	0.125–16	+
	IMP-27 (1)	0.5	4	3	+
Klebsiella oxytoca (3)	KPC (2)	8–32	3–16	8–16	+
	OXA-48 (1)	3	0.75	3	+
Klebsiella pneumoniae (28)	KPC (7)	8 to ≥32	2 to ≥32	3 to ≥32	+
	NDM (13)	3 to ≥32	2 to ≥32	4 to ≥32	+
	OXA-48 (5)	0.75–24	0.38–8	1 to ≥32	+
	OXA-181 (3)	12 to ≥32	≥32	≥32	+
Morganella morganii (3)	NDM	8 to ≥32	0.38 to ≥32	0.38–12	+
Pseudomonas putida (1)	IMP and VIM	≥32	≥32	≥32	+
Pantoea spp. (1)	KPC	12	8	24	+
Raoultella planticola (1)	KPC	2–16	0.75 to ≥32	0.75–16	+
Raoultella terrigena (2)	KPC	16	≥32	16	+
Serratia marcescens (5)	SME (4)	≥32	12 to ≥32	4 to ≥32	+
	KPC (1)	≥32	24	≥32	+
Pseudomonas aeruginosa (11)[d]	IMP (5)	≥32	≥32	≥32	+
	VIM (6)	≥32	≥32	ND	+
Carba NP test-negative carbapenemase producers[c]					
Enterobacter cloacae (1)[f]	NMC-A	≥32	3	4	−
Escherichia coli (4)	GES-5 (1)	≥32	≥32	≥32	−
	OXA-48 (3)	1.5–3	0.38–1	1.5–4	−
Klebsiella pneumoniae (13)[f]	OXA-48 (10)	0.25–12	0.094–32	0.25 to ≥32	−
	OXA-181 (3)	1–32	0.38 to ≥32	3 to ≥32	−
Proteus mirabilis (1)	IMP-27	4	4	4	−
Providencia rettgeri (1)[f]	NDM-1	≥32	≥32	16	−
Providencia stuartii (1)	NDM-1	≥32	≥32	≥32	−
Serratia marcescens (6)	GES-5 (2)	≥32	≥32	≥32	−
	SME-1 (4)	≥32	≥32	≥32	−
Pseudomonas aeruginosa (2)[d]	GES-5	≥32	≥32	ND	−

[a] *n*, number of isolates tested.

[b] Carba NP test results: −, negative (red); +, positive (yellow/orange). All the results shown in this table were obtained using 3 to 4 10-μl loopfuls resuspended in 200 μl of the lysis buffer. The same results were obtained using the original protocol (1 10-μl loopful resuspended in 100 μl of the lysis buffer), except for some OXA-48-producing isolates ($n = 15$) that were detected only by the modified protocol.

[c] Negative by PCR analysis for bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$, bla_{VIM}, bla_{IMP}, and bla_{GES}-type genes (6, 7).

[d] CLSI guidelines do not recommend ertapenem testing for *Pseudomonas*.

[e] Positive by PCR analysis for at least one of bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$, bla_{VIM}, bla_{IMP}, and bla_{GES}-type genes.

[f] Mucoid strains. Five out of 10 OXA-48-producing *K. pneumoniae* isolates were mucoid.

false-negative results, the test was repeated for these isolates with new extracts prepared with zirconia beads, as described above. However, we did not observe differences in the final results, indicating that incomplete lysis was likely not a factor in the original negative results. We do note, however, that the presence of the beads allowed for more efficient suspension of bacteria to homogeneity. A second modification was attempted by using more cells to test a more concentrated extract. In this case, 15 initially false-negative OXA-48 producers could be scored as positive (23 out of 39 OXA-48 producers were detected) (Table 1). These results increased the sensitivity and NPV of the test to 80% and 77.3%, respectively.

Overall, the Carba NP method was easy to perform, inexpensive, and, in most cases, easy to interpret, particularly on KPC and NDM producers (the color indicator turned yellow before 30 min). However, the results were less than optimal with some other carbapenemase producers, notably some harboring OXA-48-like enzymes. Blind tests performed between our laboratories showed only a few discrepancies (5 out of 74 isolates tested) and were always related to some carbapenemase producers (GES-5 or OXA-48) which were falsely negative in one laboratory but positive (faint orange) in the other lab. These blinded results highlighted the reproducibility of the test but also the problems inherent to the method, such as subjectivity in the interpretation of the results and technical interlaboratory differences. From a technical standpoint, we would recommend using more than one 10-μl loopful of bacteria in 200 μl of lysis reagent to produce a more concentrated extract (here, we used three to four loopfuls) and increase the test sensitivity. In our blind test we confirmed that this modification did not alter the specificity of the method compared to the original protocol. Notwithstanding this, we had 29 false-negative results including 2 isolates which harbored NDM (out of 33 NDM isolates) and 16 isolates harboring OXA-48-like enzymes (out of 39 OXA-48-like isolates). We also found that the Carba NP test with *S. marcescens* harboring SME-1 and *Enterobacteriaceae* harboring GES-5 were often inconsistent, being difficult to interpret with some extracts or giving false-negative results. Thus, in our hands the Carba NP test gave suboptimal results compared to those originally described (1, 2). For laboratories concerned with the widely disseminated KPC and NDM producers, however, the Carba NP test was an accurate and cost-effective method to rapidly identify potential carrier isolates which could then be further confirmed by molecular methods such as PCR; the test thus obviates the need for other less accurate and/or more time-consuming phenotypic screening methods such as the modified Hodge test or combined disk tests. Unfortunately, we found the Carba NP test unreliable for accurate identification of OXA-48-like producers, which have also emerged globally.

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We have no conflicts of interest to declare.

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