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Comparative Study of a Novel Biochemical Assay, the Rapidec Carba NP Test, for Detecting Carbapenemase-Producing *Enterobacteriaceae*

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The novel biochemical test, the Rapidec Carba NP (RCNP), was evaluated using carbapenemase- and non-carbapenemase-producing *Enterobacteriaceae* isolates. The RCNP test was compared with the Carba NP test (CNP) and the modified Hodge test. Compared to the CNP test, the RCNP test had identical sensitivity (96%) and lower specificity (93% versus 100%). The medium used to culture the isolates significantly affected test sensitivity and specificity. The RCNP test was quicker and easier to perform than the other tests.

Increasing resistance to carbapenems, which are most often the last line of therapy, is now emerging at an alarming rate in *Enterobacteriaceae* (1). Detection of carbapenemase-producing *Enterobacteriaceae* (CPE) in the clinical laboratory is of major importance for implementing infection control measures in a timely manner (2) and possibly also for therapeutic considerations (3). The molecular-based techniques used to identify CPE are time consuming and require specialized laboratory equipment and skills, whereas many of the phenotypic methods (e.g., the modified Hodge test [MHT]) have low sensitivity and specificity (as reviewed in reference 4). Thus, there is a need for a more accurate, efficient, and easy-to-use method.

The goals of this study were to assess a new, commercial assay, the Rapidec Carba NP test (RCNP) (bioMérieux, France) as a method for the detection of carbapenemases in *Enterobacteriaceae* in Israel. We compared the sensitivity and specificity of the RCNP test and the time and skill level of the clinical staff required to perform it with two tests: the Carba NP (CNP) (5, 6) and the MHT (7, 8), the method that is most commonly used for detection of carbapenemase in Israel.

We examined a collection of 98 strains that were isolated from surveillance and clinical cultures from Israeli patients from 2012 to 2014. The collection included diverse strains of the *Enterobacteriaceae* family; the species were identified using the Vitek 2 system (bioMérieux, France) (Table 1). Antimicrobial susceptibility testing for ertapenem, imipenem, and meropenem was performed by agar dilution with all study strains, and results were interpreted using the Clinical and Laboratory Standards Institute guidelines (Table 1) (9). Of the 98 strains examined, 69 were carbapenemase-producing *Enterobacteriaceae* (CPE) that tested positive by PCR for one of the following genes: bla_{KPC} (10), bla_{NDM} (11), bla_{OXA-48} (12), bla_{VIM} (13), or bla_{IMI} (14). The results of the other tests were compared to the PCR results whenever a carbapenemase gene was detected. The remaining 29 strains were non-carbapenemase-producing, carbapenem-resistant *Enterobacteriaceae* (NP-CRE), defined by a negative PCR (for the genes above and for the bla_{IMP} [15] gene) and a negative CNP test (routinely used with the CHROMagar KPC media [CHROMagar, Paris, France] in our lab, as described below).

The RCNP test was conducted according to the manufacturer's instructions, and change in color was visualized after 30 min and 2 h of incubation. CNP was performed with modifications of the originally described protocol (6, 16), as follows: A loopful (10 μl) of 24-h-old pure bacterial culture was inoculated in 200 μl of bacterial protein extraction reagent (B-Per II, catalog no. 78260; Pierce Biotechnology, IL, USA) in Eppendorf tubes. The suspensions were vortexed for 1 min, incubated at room temperature for 30 min, and centrifuged for 5 min at 10,000 × *g*. Then, 30 μl of the supernatant was mixed in 96-well microplates with a 100-μl solution of diluted 10% (vol/vol) phenol red (catalog no. P0290; Sigma-Aldrich, MO, USA) and 0.1 mM zinc sulfate (catalog no. 108883; Merck Sharp & Dohme, Darmstadt, Germany) with or without imipenem (Merck Sharp & Dohme, Chibret, France) to a final concentration of 6 mg/ml. The plates were incubated at 37°C, and a color change from red to yellow/orange was visualized after 2 h. The MHT was performed using a Mueller-Hinton agar (MH-HY) (Hylabs, Rehovot, Israel) with 10-μg meropenem disks.

In order to evaluate the effect of the culture medium on the performance of the CNP and RCNP tests, two different media were used for culturing: Mueller-Hinton E agar (MH-E) (bioMérieux, France), which is recommended by the RCNP manufacturer, and CHROMagar KPC. The latter was chosen, as it is routinely used for CRE surveillance in Israel and, also, is the media of choice for the CNP method in our lab. In addition, we added additional Mueller-Hinton media, made by a local manufacturer (i.e., MH-HY) for the RCNP test in order to evaluate the adaptability of the RCNP test. All tests were conducted after incubating the cultures on the different media at 37°C for 18 to 24 h under normal atmospheric conditions. To compare test sensitivity and specificity, we performed three separate McNemar's tests (17–19) using the samples of 69 positive and 29 negative isolates, respectively.

All tests gave an interpretable result, except in case of the RCNP

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TABLE 1 Test results for the detection of carbapenemase in *Enterobacteriaceae* using the Rapidec Carba NP, the Carba NP, and the modified Hodge tests

Carbapenemase detected (no. of isolates tested)	Species (no. of isolates tested)	No. of results by test (medium)[e]					MIC (μg/ml)[a,b]		
		Rapidec Carba NP (MH-E)	Rapidec Carba NP (MH-HY)	Carba NP (CHROMagar KPC)	Carba NP (MH-E)	Modified Hodge test	IPM	MEM	ETP
Undefined non-carbapenemase-producing carbapenem-resistant *Enterobacteriaceae* (29)[c]		27 negative, 2 positive	29 negative	29 negative	29 negative	25 negative, 4 positive			
	E. coli (9)					3	0.5 to 8	1 to >8	1 to >8
	Enterobacter cloacae (7)						2 to 8	0.5 to >8	4 to >8
	K. pneumonia (5)	2					1 to 8	0.5 to >8	0.5 to 8
	Providencia stuartii (3)						8	4	4
	Klebsiella oxytoca (1)						4	4	>8
	Citrobacter freundii (1)						4	8	8
	Enterobacter aerogenes (1)						>8	8	>8
	Proteus mirabilis (1)						>8	>8	>8
	Proteus vulgaris (1)					1	>8	<0.5	4
Carbapenemase-producing carbapenem-resistant *Enterobacteriaceae*[d]									
KPC (11)		11 positive	10 positive, 1 negative	11 positive	10 positive, 1 negative	11 positive			
	Citrobacter freundii (2)						8 to >8	8 to >8	8 to >8
	Enterobacter gergoviae (1)						>8	>8	>8
	Enterobacter aerogenes (1)		1		1		8	4	8
	Serratia marcescens (1)						8	4	>8
	Klebsiella oxytoca (1)						4	8	>8
	Citrobacter braakii (1)						>8	>8	>8
	E. coli (1)						>8	>8	>8
	K. pneumonia (1)						>8	>8	>8
	Raoultella plauticola (1)						4	8	>8
	Enterobacter cloacae (1)						4	>8	>8
NDM (19)		18 positive, 1 negative	15 positive, 4 negative	17 positive, 2 negative	13 positive, 6 negative	13 positive, 6 negative			
	Proteus rettgerii (5)			2	5	5	4 to 8	2 to 4	8 to >8
	K. pneumonia (4)					1	2 to 4	4 to >8	8 to >8
	E. coli (3)		3				4 to >8	8 to >8	8 to >8
	Proteus mirabilis (1)						>8	2	4
	Citrobacter braakii (1)						2	4	2
	Citrobacter freundii (1)						2	4	>8
	Klebsiella oxytoca (1)						2	8	>8
	Citrobacter werkmanii (1)						2	2	8
	M. morganii (1)	1	1		1		8	2	2
	Enterobacter cloacae (1)						8	>8	>8
OXA-48 (19)		18 positive, 1 negative	15 positive, 4 negative	18 positive, 1 negative	16 positive, 3 negative	18 positive, 1 negative			
	E. coli (9)	1	3	1	2	1	0.5 to 8	1 to 8	4 to >8
	K. pneumonia (7)		1		1		1 to >8	0.5to >8	4 to >8
	Enterobacter cloacae (1)						4	2	>8
	Enterobacter aerogenes (1)						2	8	4
	Klebsiella oxytoca (1)						1	4	4
VIM (15)		15 positive	15 positive	15 positive	14 positive, 1 negative	15 positive			
	Enterobacter aerogenes (5)						0.5 to 8	2 to >8	0.5 to 8
	K. pneumonia (3)						4 to >8	4 to >8	>8
	E. coli (3)				1		0.5 to 8	0.5 to 4	4 to >8
	Enterobacter cloacae (2)						1 to 8	0.5 to 4	4
	Citrobacter werkmanii (1)						>8	>8	>8
	Klebsiella oxytoca (1)						2	4	4
IMI (5)		4 positive, 1 negative	3 positive, 2 negative	5 positive	3 positive, 2 negative	4 positive, 1 negative			
	Enterobacter cloacae (5)	1	2		2	1	1 to >8	1 to >8	4 to >8

[a] ETP, ertapenem; IMP, imipenem; MEM, meropenem.

[b] When more than one isolate was examined, the range of the MICs is presented.

[c] Negative by PCR analysis for bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{VIM}, bla_{IMI}, or bla_{IMP}-type genes.

[d] Positive by PCR analysis for bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{VIM}, or bla_{IMI}-type genes.

[e] MH-E, Mueller-Hinton E agar (bioMérieux, France); MH-HY, Mueller-Hinton agar (Hylabs, Israel).

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

Performance characteristic[a]	Value by test (medium)[e]				
	Rapidec Carba NP (MH-E)[b]	Rapidec Carba NP (MH-HY)[b]	Carba NP (CHROMagar KPC)[c]	Carba NP (MH-E)[c]	Modified Hodge test
Sensitivity, % (95% CI[d])	95.6 (87.8–99.1)	84.1 (73.3–91.8)	95.6 (87.8–99)	81.2 (69.9–89.6)	88.4 (78.4–94.8)
Specificity, % (95% CI)	93.1 (77.2–99.1)	100 (88–100)	100 (88–100)	100 (88–100)	86.2 (68.3–96.1)

[a] Compared to presence of carbapenemase gene.
[b] McNemar's test for sensitivity, $P = 0.008$; specificity, $P = 0.5$.
[c] McNemar's test for sensitivity, $P = 0.008$; specificity, $P = 1$.
[d] CI, confidence interval.
[e] MH-E, Mueller-Hinton E agar (bioMérieux, France); MH-HY, Mueller-Hinton agar (Hylabs, Israel).

test when strains were grown on a CHROMagar KPC medium. After preliminary testing of 24 isolates (9 carbapenemase-negative isolates and 15 carbapenemase-producing isolates, 3 of each carbapenemase type), we concluded that carbapenemase-negative isolates were mostly indistinguishable from carbapenemase-producing isolates, probably since the chromogenic substances disrupted the interpretation of the results. Therefore, the RCNP test was deemed not applicable with the CHROMagar KPC medium and was not tested further. Using the MH-E medium, RCNP detected all but 3 (66 of 69) carbapenemase-producing isolates (sensitivity, 95.6%) and correctly identified 27 of the 29 isolates in the carbapenemase-negative isolates (specificity, 93.1%). However, when the isolates were grown on the MH-HY medium, RCNP identified only 58 of 69 positive isolates as CPE (sensitivity, 84%) and correctly identified all 29 negative isolates (specificity, 100%). Using the CHROMagar KPC medium, CNP specificity was 100% and all but 3 (66 of 69) of the CPE isolates were detected (sensitivity, 95.6%). When the isolates were grown on the MH-E medium, specificity was 100%, but the CNP test failed to detect 13 CPE isolates (sensitivity, 81.1%). The MHT detected all but 8 (61 of 69) carbapenemase-producing isolates (sensitivity, 88.4%) and correctly identified 25 of the 29 carbapenemase-negative isolates (specificity, 86.2%). The RCNP and CNP tests correctly identified all KPC and VIM producers except for one KPC-producing *Enterobacter aerogenes* isolate and one VIM-producing *Escherichia coli* isolate. Most false-negative results were observed in OXA-48-producing *E. coli* isolates and NDM-producing *Providencia rettgerii* isolates, as was previously reported (20, 21).

Increasing the inoculum of the bacteria in the CNP and RCNP tests may theoretically increase the sensitivity of the tests and thus reduce the rate of false-negative results (20, 22). However, since the inoculum is not accurately measured for these tests, this also may lead to an increase in the rate of false-positive results.

Surprisingly, we found that the choice of culture medium had a significant effect on test specificity and sensitivity (Table 2). The RCNP test had significantly superior specificity and sensitivity when the strains were grown on an MH-E medium compared to an MH-HY medium. The specificity and sensitivity of the CNP test were significantly higher when the strains were grown on the CHROMagar KPC medium compared to the MH-E medium, whereas the former was inadequate for the RCNP method. Since zinc ions are known to be essential for the activity of the metallo-β-lactamase enzymes and to affect their activity (23–25), so a possible explanation for the increased sensitivity of the RCNP test with the MH-E media may be related to the different zinc content in the MH-E and the MH-HY agar plates. Incompatibility of the CHROMagar KPC medium with the RCNP test may be explained by presence of the chromogenic substances in the plates. Since the MH-E medium is the only medium included in this study that is specifically recommended by the manufacturer for the RCNP test, these findings highlight the critical importance of strict adherence to the manufacturer's instructions, including the choice of commonly used bacteriological media (e.g., Muller-Hinton media).

The MHT was less sensitive than both the RCNP test using MH-E and the CNP test using CHROMagar KPC. Also, the MHT had lower specificity than all other tests (Table 2).

Although we did not found correlation between the isolate MICs and the ability of the tests to detect carbapenemases in the examined isolates, we noticed that *E. coli* and *Klebsiella pneumoniae* strains that carried the $bla_{\text{OXA-48}}$ gene showed low hydrolysis activity, which made the interpretation of the results more difficult to analyze. This is probably due to the weak hydrolysis activity of the OXA-48 enzyme that is known to be the most difficult carbapenemase to identify (12, 21).

This study's strength was the examination of a wide diversity of species and enzymes, notably those harboring the OXA-48-like enzyme and the *Providencia* species strains harboring the bla_{NDM} gene, that are known to be difficult to interpret and present inconsistent or false-negative results using phenotypic methods. For these isolates, the RCNP test performed better than all other tests, with one false-negative (*Morganella morganii*) among 19 isolates harboring the bla_{NDM} gene and one false-negative (*E. coli*) among 19 isolates harboring the $bla_{\text{OXA-48-like}}$ gene. Thus, for laboratories concerned with the widely encountered KPC and NDM producers, the RCNP test would provide excellent sensitivity and specificity. In addition, the results using the RCNP test were apparent after 30 min of incubation, compared to 2 h with the CNP test. Moreover, the RCNP test required less technical skill and special equipment than the other tests, since its procedure did not included centrifugation and preparation of solutions and antibiotics.

Although there were no significant differences in sensitivity and specificity between the CNP test and the RCNP test using their preferred media, we found that, overall, the RCNP test, if used with the MH-E agar plates, was easily performed and accurate and had a faster turnaround time than other phenotypic screening methods used to identify isolates harboring carbapenemases.

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