

Evaluation of the Rapidec Carba NP Test Kit for Detection of Carbapenemase-Producing Gram-Negative Bacteria

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Recently, bioMérieux, France, introduced the Rapidec Carba NP test kit for rapid detection of carbapenemase-producing Gram-negative bacteria. This kit was evaluated in this study, and we report sensitivity, specificity, and positive and negative predictive values of 92.6%, 96.2%, 95.83%, and 92.6%, respectively. The test was easy to perform and interpret and relatively inexpensive (\$5/Rs 300 per test) and provides a practical solution for early detection of carbapenemase-producing, multidrug-resistant Gram-negative bacteria.

Carbapenems are the most effective antimicrobial agents against Gram-negative bacteria and have a broad spectrum of antibacterial activity against members of the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* complex, etc. They are generally used as last-resort drugs for the treatment of infections caused by multidrug-resistant bacteria (1). Mechanisms of resistance to carbapenems include production of β -lactamases, efflux pumps, and mutations that alter the expression and/or function of porins and penicillin-binding proteins. Carbapenemases are specific β -lactamases with the ability to hydrolyze carbapenems, and their production appears to be the most important cause of carbapenem resistance. It is well documented that there is widespread distribution of carbapenemases in Gram-negative bacteria (1). An increasing number of Ambler class A carbapenemases (e.g., KPC and GES enzymes), class B metallo- β -lactamases (e.g., VIM, IMP, and NDM β -lactamases), and class D carbapenemases (e.g., OXA-23, -48, -51, and -143) have recently emerged (2). Carbapenem-resistant Gram-negative bacilli remain a formidable threat, as few antimicrobial agents are reliably active and very little is expected to be available in the near future to combat this situation.

The detection of carbapenemases in clinical microbiology labs is challenging because phenotypic tests like the modified Hodge test and triple-disc test (Rosco Diagnostics, Denmark) are time-consuming and difficult to interpret. PCR-based molecular methods, UV spectrophotometry, and MALDI-TOF MS (matrix-assisted laser desorption ionization–time of flight mass spectrometry) assays have overall good sensitivity and specificity but require trained personnel and good infrastructure and are expensive (3).

Recently bioMérieux (La Balme-les-Grottes, France) introduced the Rapidec Carba NP (carbapenemase Nordmann-Poirel) kit to detect carbapenemases from bacterial colonies grown on recommended selective or nonselective agar plates. The manufacturer claims detection of carbapenemase producing bacteria in 30 min to 2 h, with sensitivity and specificity of 97.8% compared with molecular techniques (www.biomerieux-diagnostics.com/sites/clinic/files/9308689-002-gb-a-rapidec-carba-np.pdf). This kit was evaluated by Poirel and Nordmann (4) and Dortet et al. (5) in 2015 using precharacterized bacterial isolates at the Emerging Antibiotic Resistance Unit, University of Fribourg, Switzerland, and the National Reference Center for Antibiotic Resistance, France, respectively. However, to the best of our knowledge, no indepen-

dent field trial of this kit has been reported by any laboratory worldwide to date, and this study was planned with the aim of evaluating the field efficacy of the Rapidec Carba NP kit in a resource-limited bacteriology laboratory of a developing country like India.

The strains used in this study were collected from various clinical samples of patients admitted to L.L.R & Associated Hospitals (10 hospitals with different superspecialties), GSVM Medical College, Kanpur, and District Hospital, Kanpur, India. All isolated nonduplicate strains were initially screened for carbapenem sensitivity by the disc diffusion method; nonsusceptibility to carbapenems was confirmed by MIC estimation using E strips, and the results were interpreted using the Clinical and Laboratory Standards Institute guidelines (6). Environmental and surveillance samples were not included in our study. A panel of 100 bacterial isolates were selected for testing: 50 containing different classes of carbapenemases (NDM, KPC, VIM, IMP, and OXA-48, all confirmed by PCR [7] and sequence analysis) and 50 non-carbapenemase producers, of which 25 were sensitive to carbapenem and 25 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 confirmation kit; Rosco Diagnostics, Denmark) were also negative; furthermore, to rule out the presence of any carbapenemase activity, carbapenem hydrolysis was performed spectrophotometrically as described earlier (8), and it was concluded that the carbapenem resistance observed in this group was due to some other mechanism (Table 1). The clonality of the strains of the family *Enterobacteriaceae* was determined by enterobacterial repetitive intergenic consensus (ERIC) PCR, whereas for nonfermenters, repetitive extragenic palindromic (REP) PCR was performed.

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TABLE 1 Results of the Rapidec Carba NP test with carbapenemase-producing and non-carbapenemase-producing Gram-negative bacteria

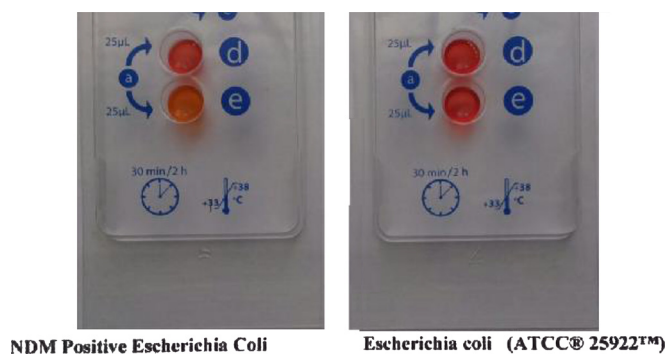
Species (no. of isolates tested)	Carbapenemase detected (no. of isolates tested)	MIC ($\mu\text{g/ml}$) of imipenem	Rapidec Carba NP test result
Carbapenemase-producing bacteria, Rapidec Carba NP positive (46) ^a			
<i>Escherichia coli</i> (23)	NDM (12)	4–128	+
	OXA-48 (6)	1–8	+
	KPC (2)	16–32	+
	VIM (2)	32	+
	IMP (1)	16	+
<i>Pseudomonas aeruginosa</i> (10)	NDM (5)	4–64	+
	IMP (3)	8–32	+
	KPC (2)	8–16	+
<i>Acinetobacter baumannii</i> (5)	NDM (2)	16–32	+
	KPC (2)	4–8	+
	IMP (1)	8	+
<i>Klebsiella pneumoniae</i> (3)	NDM (1)	32	+
	KPC (2)	8–16	+
<i>Proteus mirabilis</i> (2)	NDM (1)	32	+
	KPC (1)	32	+
<i>Citrobacter freundii</i> (1)	NDM (1)	16	+
<i>Enterobacter cloacae</i> (1)	OXA-48 (1)	8	+
<i>Stenotrophomonas maltophilia</i> (1)	NDM (1)	8	+
Carbapenemase-producing bacteria, Rapidec Carba NP negative (4) ^a			
<i>Escherichia coli</i> (3)	OXA-48 (2)	4–8	–
	IMP (1)	4	–
<i>Klebsiella pneumoniae</i> (1)	OXA-48 (1)	4	–
Non-carbapenemase-producing, carbapenem-resistant bacteria, Rapidec Carba NP positive (2) ^b			
<i>Escherichia coli</i> (1)		4	+
<i>Pseudomonas aeruginosa</i> (1)		8	+
Non-carbapenemase-producing bacteria, Rapidec Carba NP negative (48) ^b			
<i>Escherichia coli</i> (19)		0.06–8	–
<i>Pseudomonas aeruginosa</i> (9)		0.06–32	–
<i>Acinetobacter baumannii</i> (6)		0.12–16	–
<i>Klebsiella pneumoniae</i> (8)		0.06–32	–
<i>Proteus mirabilis</i> (2)		0.12–4	–
<i>Citrobacter freundii</i> (2)		0.06–4	–
<i>Enterobacter cloacae</i> (2)		0.12–4	–

^a PCR- and imipenem hydrolysis-positive strains.

^b PCR- and imipenem hydrolysis-negative strains.

The test was performed as recommended by the manufacturer. A loopful (10- μl) of a bacterial colony was picked up from overnight-incubated Mueller-Hinton agar plates (bioMérieux) and mixed into API suspension medium (provided with kit); the bacterial suspension was then transferred to wells in the test strip and incubated at 37°C. Visual reading of the test strip was done after 30 min and after 2 h, if necessary. A positive test corresponded to a color change from red to yellow-orange, whereas a red color indicated a negative result (Fig. 1). The test strips were read by two independent blinded readers to determine interrater reliability. Any discrepancy between the two readings was resolved by an independent blinded third reader, whose reading was taken as final.

The kit detected carbapenemase activity in 46 out of 50 PCR-confirmed strains. However, it could not detect carbapenemase activity in 3 OXA-48-positive strains (two *Escherichia coli* isolates [nonclonal] and one *Klebsiella pneumoniae* isolate) and one IMP-positive *Escherichia coli* strain. False-positive results were also obtained with two carbapenemase-negative carbapenem-resistant



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FIG 1 Rapidec Carba NP test results. The yellow-orange color in well E with NDM-positive *Escherichia coli* indicates a positive result, whereas the red color with *Escherichia coli* ATCC 25922 indicates a negative result.

TABLE 2 Diagnostic accuracy of Rapidec Carba NP

Statistical index	Value (%)	95% confidence interval (%)
Sensitivity	92.00	80.77–97.78
Specificity	96.00	86.29–99.51
Positive predictive value	95.83	85.75–99.49
Negative predictive value	92.31	81.46–97.86

strains (one *Escherichia coli* and one *Pseudomonas aeruginosa* isolate). None of the carbapenem-sensitive bacteria were positive. Overall, the kit showed good efficacy (Table 2).

To date, the Rapidec Carba NP kit had been evaluated in only two studies. Poirel and Nordmann (4) tested this kit with precharacterized strains and documented a sensitivity and specificity of 96%; they also reported false-negative results with group D carbapenemases, which is in accordance with the fact that group D carbapenemases are known to lack significant carbapenemase activity. In a similar study from the National Reference Center for Antibiotic Resistance, France, Dortet et al. (5) documented a sensitivity of 99% and specificity of 100%.

To conclude, the Rapidec Carba NP test was simple, easy to perform and interpret, and relatively inexpensive (\$5/Rs 300 per test) and in most cases gave results in <30 min. The kit was found to be well suited for rapid detection of NDM, the most common carbapenemase isolated in the Indian subcontinent (9). The kit can also be used to screen the strains before expensive molecular tests, like PCR and gene sequencing, are performed for identification of the carbapenemase genes. This kit has a high degree of sensitivity and specificity and provides a practical solution for early detection of carbapenemase-producing, multidrug-resistant Gram-negative bacteria in peripheral microbiological laboratories. Further, it will help in implementation of infection prevention and control measures, e.g., patient isolation and quarantine, to limit the spread of carbapenemase producers and avoid outbreaks in health care settings.

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We have no conflicts of interest to declare.

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