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Evaluation of the RAPIDEC® CARBA NP, the Rapid CARB Screen® and the Carba NP test for biochemical detection of carbapenemase-producing Enterobacteriaceae

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Objectives: The objective of this study was the evaluation of the performance of two commercially available biochemical tests for the rapid detection of carbapenemase-producing Enterobacteriaceae compared with a home-made technique.

Methods: A collection of 150 enterobacterial isolates, including 132 isolates with decreased susceptibility to at least one carbapenem molecule, were tested for carbapenemase activity using the RAPIDEC® CARBA NP (bioMérieux), the Rapid CARB Screen® (Rosco Diagnostica) and the home-made Carba NP test. This strain collection included 55 non-carbapenemase producers, 21 KPC producers, 21 NDM producers, 17 VIM producers, 11 IMP producers, 16 OXA-48 producers and 9 OXA-48-like producers (OXA-162, OXA-181, OXA-204, OXA-232 and OXA-244).

Results: The RAPIDEC® CARBA NP detected all carbapenemase producers except a single OXA-244 producer. Using the Rapid CARB Screen®, one KPC-2, two NDM-1, one OXA-48 and five OXA-48 variant producers gave equivocal results and one OXA-244 producer was not detected. Using the Carba NP test, the same OXA-244 producer was not detected and one OXA-181 producer and one OXA-244 producer gave equivocal results. Sensitivity and specificity were 99% (95% CI 94.3%–99.8%) and 100% (95% CI 93.5%–100%), respectively, for the RAPIDEC® CARBA NP test, 89.5% (95% CI 81.7%–94.2%) and 70.9% (95% CI 57.9%–81.2%) for the Rapid CARB Screen® and 96.8% (95% CI 91.1%–98.9%) and 100% (95% CI 93.5%–100%) for the Carba NP test. The impact of the use of an adequate bacterial inoculum for obtaining the optimal performance with the RAPIDEC® CARBA NP was noted.

Conclusions: The RAPIDEC® CARBA NP possesses the best performance for rapid and efficient detection of carbapenemase-producing Enterobacteriaceae.

Introduction

During the last decade, decreased susceptibility to carbapenems has been increasingly reported worldwide in Gram-negative organisms, including Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp.[1] Carbapenem resistance may result either from the association of a decrease in outer membrane permeability with overexpression of β-lactamases possessing very weak carbapenemase activity, or from β-lactamases with strong hydrolytic activity towards carbapenems, i.e. carbapenemases.[2] A variety of carbapenemases have been reported in Enterobacteriaceae, such as KPC (Ambler class A), metallo-β-lactamases (MBLs) of the VIM, IMP and NDM types (Ambler class B) and OXA-48-type enzymes (Ambler class D).[3] Usually carbapenemase-producing Enterobacteriaceae (CPE) are resistant to most β-lactams and non-β-lactam antibiotics, resulting in MDR and even pandrug-resistant isolates.[4] As a consequence, the spread of these carbapenemase producers represents a serious threat to public health, especially since only a few novel antibiotics are expected in the near future.[5] Rapid and efficient detection of CPE is becoming a major issue in limiting the spread of these highly resistant bacteria. Recently, several methods have been developed to detect CPE.

These methods are based on: (i) the detection of carbapenem-hydrolysing activity by monitoring the colour change of a pH indicator (the Carba NP test[6] and its derivatives[7,8]) or by following enzymatic degradation products by MALDI-TOF protocols;[9] or (ii) molecular techniques to detect the main known carbapenemase genes.[10]

Considering the importance of accurately detecting CPE, our aim was to evaluate the performance of two biochemical rapid tests recently commercialized, the RAPIDEC® CARBA NP and the Rapid CARB Screen®, for detection of CPE, compared with the home-made Carba NP test, which is the reference test for this biochemical detection.[6]

Materials and methods

Strain collection

A total of 150 strains of enterobacteria were used to evaluate the performance of the RAPIDEC® CARBA NP (bioMérieux, La Balme-les-Grottes, France) and the Rapid CARB Screen® (Rosco Diagnostica, Taastrup, Denmark) in comparison with the Carba NP test.[6,11] All strains had previously been characterized for their β-lactamase content at the molecular level.[6] This collection included 55 non-carbapenemase producers (Table 1), among which 37 have decreased susceptibility to at least one carbapenem molecule (imipenem, meropenem, ertapenem) and 95 CPE. These CPE were of various enterobacterial species, isolated from various clinical samples (blood cultures, urine, sputum etc.) from our own strain collection of global origin, and included 21 KPC producers, 21 NDM producers, 17 VIM producers, 11 IMP producers, 16 OXA-48 producers and 9 OXA-48-like producers (Table 1).

Antibiotic susceptibility testing

MICs of carbapenems were determined using the Etest® (bioMérieux) and results were recorded according to EUCAST guidelines, as updated in 2015 (http://www.eucast.org). Clinical carbapenem breakpoints for susceptibility/resistance were ≤2/>8 mg/L for imipenem and meropenem and ≤0.5/>1 mg/L for ertapenem.

RAPIDEC® CARBA NP

The RAPIDEC® CARBA NP was performed using a standardized inoculum (a full 10 μL loop of bacterial colonies), which is critical for test reliability. The test was performed on bacterial colonies recovered from Trypticase soy agar (Oxoid, Dardilly, France). The bacterial inoculum was transferred from the loop into the RAPIDEC® CARBA NP using the plastic stick provided in the kit. The RAPIDEC® CARBA NP results were interpreted using the manufacturer's interpretation guidelines (Figure S1A, available as Supplementary data at *JAC* Online).

Rapid CARB Screen®

The Rapid CARB Screen® test was performed on bacterial colonies recovered from Trypticase soy agar (Oxoid) and interpreted (Figure S1B) according to the manufacturer's instructions. As recommended, if an orange or light yellow colour appeared in the tube containing the imipenem tab, the test was repeated using a higher inoculum.

Carba NP test

The updated version of the Carba NP test was used and interpreted (Figure S1C) as previously described.[11] The test was performed on bacterial colonies recovered from Trypticase soy agar (Oxoid).[11] Reading was performed within 2 h.

Results and discussion

Performance of the RAPIDEC® CARBA NP, the Rapid CARB Screen® and the Carba NP test

Preliminary experiments done with the RAPIDEC® CARBA NP identified a critical impact of the bacterial inoculum on the performance of the test. Indeed, an insufficient bacterial inoculum may yield false positive results (data not shown). Respecting the turbidity positive control for the bacterial inoculum is therefore critical. Accordingly, the bacterial inoculum has been standardized as a full 10 μL loop of bacterial colonies recovered from the Trypticase soy agar.

The RAPIDEC® CARBA NP, the Rapid CARB Screen® and the Carba NP test results for the detection of CPE and non-carbapenemase producers are detailed in Table 2. Considering equivocal results as invalid (false positive or false negative), sensitivity and specificity were 99% (95% CI 94.3%–99.8%) and 100% (95% CI 93.5%–100%), respectively, for the RAPIDEC® CARBA NP, 89.5% (95% CI 81.7%–94.2%) and 70.9% (95% CI 57.9%–81.2%) for the Rapid CARB Screen® and 96.8% (95% CI 91.1%–98.9%) and 100% (95% CI 93.5%–100%) for the Carba NP test.

Most of the CPE isolates yielded positive results (after 2 h of incubation) with the three tests (Table 1). However, using the Rapid CARB Screen®, a single KPC-2, two NDM-1, a single OXA-48, two OXA-181, two OXA-232 producers and a single OXA-244 producer gave equivocal results (weakly orange) and a single OXA-244 producer gave a negative result (Table 1). As recommended by the manufacturer, the isolates giving equivocal results were retested using a higher inoculum of bacteria (data not shown), leading to positive results for the KPC-2 producer and for a single NDM-1 producer. Despite this increased inoculum, one NDM-1 producer, one OXA-48 producer and the five OXA-48-like producers remained equivocal with the Rapid CARB Screen®. In addition, this test yielded equivocal results for 29.1% (16/55) of the non-carbapenemase producers (Table 1), which could not be resolved using a higher inoculum (the results for the 16 non-carbapenemase-producing isolates remained equivocal).

The Carba NP test yielded equivocal results for a single OXA-181-producing isolate and a single OXA-244 producer (Table 1) and was unable to detect a single OXA-244 producer (Table 1). On the other hand, the RAPIDEC® CARBA NP was able to detect all CPE except a single OXA-244 producer. Strikingly, the same OXA-244-producing *Escherichia coli* isolate gave negative/equivocal results with all three assays. The lack of detection of carbapenemase activity might be explained by: (i) the lower hydrolytic activity of OXA-244 compared with OXA-48; and/or (ii) the lower level of expression of the enzyme, since the $bla_{OXA-244}$ gene was chromosomally located as a single copy in that strain (L. Dortet, unpublished data). In contrast, for the OXA-244-producing *E. coli* isolate that gave positive results with the RAPIDEC® CARBA NP, the $bla_{OXA-244}$ gene was carried by a plasmid.

Overall, the RAPIDEC® CARBA NP possessed the best performance, with 99% (95% CI 94.3%–99.8%) sensitivity and 100% (95% CI 93.5%–100%) specificity. This performance is in accordance with the manufacturer's specifications [sensitivity 97.8% (95% CI 93.7%–99.2%) and specificity 97.8%

Table 1. Results of the RAPIDEC® CARBA NP, the Rapid CARB Screen and the Carba NP test with carbapenemase- and non-carbapenemase-producing Enterobacteriaceae by using a collection of carbapenemase and non-carbapenemase producers

	Name	Species	β-Lactamase content[a]	RAPIDEC® CARBA NP[b]	Rapid CARB Screen®[c]	Carba NP test[d]	MIC (mg/L)		
							IPM	ETP	MEM
Ambler class A carbapenemases									
KPC type	1	*E. coli*	**KPC-2**	++	+	++	1	>32	3
	2	*E. coli*	**KPC-2**	++	+	++	0.5	0.5	0.5
	3	*E. coli*	**KPC-2**+TEM-1+OXA-9	++	+	++	2	1.5	1
	4	*E. coli*	**KPC-2**+CTX-M-9+TEM-1	++	+	++	4	4	2
	1	*Klebsiella pneumoniae*	**KPC-2**+SHV-11+TEM-1+CTX-M-2	++	+	++	16	24	32
	2	*K. pneumoniae*	**KPC-2**+SHV-11+TEM-1+CTX-M-2+OXA-9	++	+	++	>32	>32	>32
	3	*K. pneumoniae*	**KPC-2**+SHV-11+CTX-M-15	++	+	++	16	>32	>32
	4	*K. pneumoniae*	**KPC-2**+TEM-1+SHV-1+CTXM-15	++	+	++	4	4	32
	5	*K. pneumoniae*	**KPC-2**+SHV-11+TEM-1+SHV-12+OXA-9	++	+	++	4	24	2
	6	*K. pneumoniae*	**KPC-2**+SHV-11	++	+	++	>32	>32	>32
	7	*K. pneumoniae*	**KPC-2**+SHV-11+TEM-1	++	+	++	4	6	8
	8	*K. pneumoniae*	**KPC-3**	+	+	++	8	12	2
	9	*K. pneumoniae*	**KPC-3**+SHV-11+OXA-9+TEM-1	++	+	++	8	>32	8
	1	*Enterobacter cloacae*	**KPC-2**	++	+	++	1	1.5	0.75
	2	*E. cloacae*	**KPC-2**+TEM-1	++	+	++	24	>32	16
	3	*E. cloacae*	**KPC-2**+TEM-1+OXA-1	++	+	++	4	6	2
	4	*E. cloacae*	**KPC-2**+TEM-1+SHV-11	++	+	++	2	4	1.5
	5	*E. cloacae*	**KPC-2**+TEM-3	++	+	++	2	2	1
	1	*Citrobacter freundii*	**KPC-2**+TEM-1	++	+	++	8	1.5	3
	1	*Serratia marcescens*	**KPC-2**+TEM-1+SHV-12	++	+	++	>32	>32	>32
	2	*S. marcescens*	**KPC-2**+TEM-1	++	+/−	++	>32	>32	>32
Ambler class B carbapenemases									
NDM type	1	*E. coli*	**NDM-1**+OXA-1+OXA-10+CMY-16+TEM-1	++	+	++	1	3	1
	2	*E. coli*	**NDM-1**+OXA-1+TEM-1	++	+	++	3	3	2
	3	*E. coli*	**NDM-1**+CTX-M-15+TEM-1	++	+	++	6	32	16
	4	*E. coli*	**NDM-1**+OXA-1+OXA-2+CTX-M-15+TEM-1	++	+	++	4	>32	8
	5	*E. coli*	**NDM-1**+CTX-M-15+TEM-1	++	+	++	16	>32	16
	6	*E. coli*	**NDM-4**+CTX-M-15+OXA-1	++	+	++	>32	>32	>32
	7	*E. coli*	**NDM-4**+CTX-M-15+CMY-6	++	+	++	>32	>32	>32
	8	*E. coli*	**NDM-5**+TEM-1+CTX-M-15	++	+	++	>32	>32	>32
	9	*E. coli*	**NDM-6**+CTX-M-15+OXA-1	++	+	++	6	32	8
	10	*E. coli*	**NDM-7**	++	+	++	4	16	3
	1	*K. pneumoniae*	**NDM-1**+CTX-M-15+SHV-11+OXA-1	++	+	++	2	8	3
	2	*K. pneumoniae*	**NDM-1**+CTX-M-15+CMY-4+OXA-1	++	+	++	>32	>32	>32
	3	*K. pneumoniae*	**NDM-1**+CTX-M-15+OXA-1+OXA-9+TEM-1+SHV-28+SHV-11	++	+	++	>32	>32	>32

	4	*K. pneumoniae*	**NDM-1**+OXA-1+SHV-11	++	+	++	1.5	6	2
	5	*K. pneumoniae*	**NDM-1**+OXA-1+CTX-M-15+TEM-1+SHV-28+OXA-9+CMY-6	++	+	++	1	8	4
	6	*K. pneumoniae*	**NDM-1**+TEM-1+CTX-M-15+SHV-12+OXA-9	++	+	++	1.5	8	1.5
	7	*K. pneumoniae*	**NDM-1**+TEM-1+CTX-M-15+SHV-12+OXA-9	++	+	++	4	8	16
	8	*K. pneumoniae*	**NDM-1**+TEM-1+CTX-M-15+SHV-11+OXA-1	++	+	++	2	>32	4
	1	*Providencia stuartii*	**NDM-1**+OXA-1+CMY-6+TEM-1	++	+/−	+	12	0.38	1.5
	1	*Providencia rettgeri*	**NDM-1**+CTX-M-15	++	+/−	+	3	0.5	1.5
	1	*Salmonella enterica*	**NDM-1**+CTX-M-15+TEM-1+OXA-1+OXA-9+OXA-10	++	+	++	4	6	3
VIM type	1	*E. coli*	**VIM-1**+CTX-M-3	++	+	++	1.5	0.38	0.5
	2	*E. coli*	**VIM-1**+CMY-13	++	+	++	3	1.5	1
	3	*E. coli*	**VIM-4**	++	+	++	8	4	3
	1	*K. pneumoniae*	**VIM-1**+SHV-5	++	+	++	>32	>32	>32
	2	*K. pneumoniae*	**VIM-1**	++	+	++	>32	>32	>32
	3	*K. pneumoniae*	**VIM-1**+SHV-12	++	+	++	>32	>32	>32
	4	*K. pneumoniae*	**VIM-1**	++	+	++	>32	>32	>32
	5	*K. pneumoniae*	**VIM-1**+SHV-5	++	+	++	4	2	2
	6	*K. pneumoniae*	**VIM-1**+TEM-1+SHV-5	++	+	++	>32	>32	>32
	7	*K. pneumoniae*	**VIM-1**+SHV-5	++	+	++	>32	>32	>32
	8	*K. pneumoniae*	**VIM-1**+CTX-M-3	++	+	++	1	0.5	1
	9	*K. pneumoniae*	**VIM-1**+SHV-5	++	+	++	0.5	4	0.38
	10	*K. pneumoniae*	**VIM-19**+CTX-M-3+TEM-1+SHV-1	++	+	++	8	16	4
	1	*E. cloacae*	**VIM-1**+SHV-70	++	+	++	1	0.38	0.5
	2	*E. cloacae*	**VIM-4**+CTX-M-15+TEM-1+SHV-31	++	+	++	3	2	1
	1	*C. freundii*	**VIM-2**+TEM-1 +	++	+	++	2	2	0.75
	2	*C. freundii*	**VIM-2**+TEM-1+OXA-9+OXA-10	++	+	++	1.5	4	0.5
IMP type	1	*E. coli*	**IMP-1**	++	+	++	0.5	3	0.5
	2	*E. coli*	**IMP-8**+SHV -12	++	+	++	6	8	3
	1	*K. pneumoniae*	**IMP-1**	++	+	++	1.5	3	1
	2	*K. pneumoniae*	**IMP-1**+TEM-15	++	+	++	8	3	2
	3	*K. pneumoniae*	**IMP-1**+TEM-1+CTX-M-15	++	+	++	1.5	4	2
	4	*K. pneumoniae*	**IMP-1**+SHV-5	++	+	++	1	2	8
	5	*K. pneumoniae*	**IMP-8**	++	+	++	1	1	0.5
	6	*K. pneumoniae*	**IMP-8**+SHV -12	++	+	++	0.5	0.5	0.5
	1	*E. cloacae*	**IMP-8**	++	+	++	1.5	1	1
	2	*E. cloacae*	**IMP-8**+SHV-12	++	+	++	0.75	0.5	0.5
	1	*S. marcescens*	**IMP-11**	++	+	++	8	>32	2
Ambler class D carbapenemases									
OXA-48	1	*E. coli*	**OXA-48**+CTX-M-15	+	+	++	3	16	1
	2	*E. coli*	**OXA-48**+CTX-M-15	++	+	++	0.5	0.75	0.12
	3	*E. coli*	**OXA-48**+CTX-M-15	+	+	+	0.38	1.5	0.19
	4	*E. coli*	**OXA-48**+CTX-M-24+TEM-1	+	+	+	0.25	0.5	0.19
	1	*K. pneumoniae*	**OXA-48**	+	+	++	0.5	2	0.5

Continued

Table 1. *Continued*

	Name	Species	β-Lactamase content[a]	RAPIDEC® CARBA NP[b]	Rapid CARB Screen®[c]	Carba NP test[d]	MIC (mg/L)		
							IPM	ETP	MEM
	2	*K. pneumoniae*	**OXA-48** + TEM-1	++	+	+	0.38	1	0.5
	3	*K. pneumoniae*	**OXA-48** + CTX-M-15	++	+	++	2	3	2
	4	*K. pneumoniae*	**OXA-48**	++	+/−	++	1	4	1
	5	*K. pneumoniae*	**OXA-48**	++	+	++	1	4	1
	6	*K. pneumoniae*	**OXA-48**	+	+	++	>32	>32	>32
	7	*K. pneumoniae*	**OXA-48** + SHV-11	+	+	++	0.5	0.75	0.25
	1	*E. cloacae*	**OXA-48** + TEM-1 + CTX-M-15 + OXA-1	+	+	++	0.5	2	0.5
	2	*E. cloacae*	**OXA-48** + TEM-1 + CTX-M-15 + OXA-1	+	+	++	1	16	1.5
	1	*Citrobacter koseri*	**OXA-48**	++	+	++	0.38	2	0.38
	2	*C. koseri*	**OXA-48** + TEM-1	++	+	++	0.75	2	0.38
	1	*C. freundii*	**OXA-48** + SHV-12 + TEM-1	++	+	++	1	3	0.75
OXA-162	1	*K. pneumoniae*	**OXA-162** + TEM-1 + SHV-11	+	+	++	4	8	1
OXA-181	1	*K. pneumoniae*	**OXA-181** + SHV-11 + CTXM-15 + OXA-1	+	+/−	++	0.5	2	0.5
	1	*E. coli*	**OXA-181**	+	+	+	0.5	1.5	0.5
	2	*E. coli*	**OXA-181**	+	+/−	+/−	0.75	2	0.5
OXA-204	1	*K. pneumoniae*	**OXA-204** + CMY-4	+	+	++	0.5	2	0.5
OXA-232	1	*E. coli*	**OXA-232** + CTX-M-15 + OXA-1	+	+/−	+	>32	>32	>32
	1	*K. pneumoniae*	**OXA-232** + SHV-1 + TEM-1 + CTX-M-15 + OXA-1	+	+/−	++	3	>32	12
OXA-244	1	*E. coli*	**OXA-244** + TEM-1 + CMY-2	+	−	+/−	0.5	2	0.5
	2	*E. coli*	**OXA-244** + TEM-1 + CMY-2	−	+/−	−	0.5	1.5	0.5
Non-carbapenemase producers									
WT	ATCC 700603	*K. pneumoniae*	SHV-11	−	−	−	0.06	0.06	0.06
acquired CPase	1	*E. coli*	DHA-1	−	+/−	−	0.12	0.02	0.02
	1	*E. coli*	ACC-1	−	+/−	−	0.12	0.12	0.12
	1	*K. pneumoniae*	DHA-2	−	−	−	0.12	0.5	0.12
	1	*Proteus mirabilis*	ACC-1	−	+/−	−	0.25	0.12	0.12
ESBL	1	*E. coli*	CTX-M-1	−	−	−	0.12	0.12	0.12
	1	*E. coli*	CTX-M-3	−	−	−	0.12	0.12	0.12
	1	*K. pneumoniae*	CTX-M-3	−	−	−	0.12	0.12	0.12
	1	*E. coli*	CTX-M-14	−	+/−	−	0.12	0.12	0.12
	2	*E. coli*	CTX-M-14	−	−	−	0.12	0.12	0.12
	1	*K. pneumoniae*	CTX-M-14	−	−	−	0.12	0.12	0.12
	1	*E. coli*	CTX-M-15	−	−	−	0.12	0.12	0.12
	2	*E. coli*	CTX-M-15	−	−	−	0.12	0.12	0.12
	1	*K. pneumoniae*	CTX-M-15	−	−	−	0.12	0.12	0.12

	2	*K. pneumoniae*	CTX-M-15	−	−	−	0.12	0.12	0.12
	3	*K. pneumoniae*	CTX-M-15	−	−	−	0.12	0.12	0.12
	1	*E. cloacae*	CTX-M-15	−	−	−	0.12	0.12	0.12
	1	*E. cloacae*	VEB-1	−	−	−	0.12	0.12	0.12
CPase + impermeability	1	*E. coli*	↑↑↑ Case	−	−	−	16	>32	2
	1	*E. cloacae*	↑↑↑ Case	−	+/−	−	0.12	1	0.12
	2	*E. cloacae*	↑↑↑ Case	−	−	−	0.12	1	0.12
	3	*E. cloacae*	↑↑↑ Case	−	−	−	0.25	4	0.25
	4	*E. cloacae*	↑↑↑ Case	−	+/−	−	4	1.5	0.75
	5	*E. cloacae*	↑↑↑ Case	−	−	−	0.19	1.5	0.12
	6	*E. cloacae*	↑↑↑ Case	−	−	−	0.25	1	0.12
	7	*E. cloacae*	↑↑↑ Case	−	−	−	0.75	2	0.12
	8	*E. cloacae*	↑↑↑ Case	−	−	−	0.19	1	0.12
	9	*E. cloacae*	↑↑↑ Case	−	+/−	−	0.25	1	0.12
	10	*E. cloacae*	↑↑↑ Case	−	+/−	−	0.25	1.5	0.12
	11	*E. cloacae*	↑↑↑ Case	−	−	−	0.38	2	0.12
	12	*E. cloacae*	↑↑↑ Case	−	+/−	−	0.5	1.5	0.25
	13	*E. cloacae*	↑↑↑ Case	−	+/−	−	1	3	0.5
	14	*E. cloacae*	↑↑↑ Case	−	−	−	1	2	0.5
	1	*Enterobacter aerogenes*	↑↑↑ Case	−	+/−	−	1	4	0.75
	1	*Morganella morganii*	↑↑↑ Case	−	−	−	1.5	0.02	0.12
ESBL + impermeability	1	*E. coli*	CTX-M-15	−	−	−	2	4	1
	1	*K. pneumoniae*	CTX-M-15 + SHV-1	−	+/−	−	1	>32	4
	2	*K. pneumoniae*	CTX-M-15 + TEM-1 + SHV-1	−	+/−	−	1.5	>32	4
	3	*K. pneumoniae*	CTX-M-15 + TEM-1 + SHV-1	−	−	−	0.25	1	1
	4	*K. pneumoniae*	CTX-M-15 + SHV-11	−	−	−	1.5	>32	6
	5	*K. pneumoniae*	CTX-M-15 + SHV-28 - TEM-1	−	+/−	−	8	>32	4
	6	*K. pneumoniae*	TEM-1 + SHV-28	−	−	−	1	4	1
	7	*K. pneumoniae*	CTX-M-15 + TEM-1 + SHV-11	−	−	−	3	>32	6
	8	*K. pneumoniae*	CTX-M-15 + TEM-1 + SHV-11	−	−	−	0.25	1	1
	9	*K. pneumoniae*	CTX-M-15 + TEM-1 + SHV-11	−	−	−	6	>32	>32
	10	*K. pneumoniae*	CTX-M-15 + TEM-1 + SHV-12	−	−	−	0.75	>32	3
	11	*K. pneumoniae*	CTX-M-15 + TEM-1 + SHV-11	−	−	−	1	24	0.5
	12	*K. pneumoniae*	CTX-M-15 + TEM-1 + SHV-1 + OXA-1	−	+/−	−	2	4	1
ESBL + CPase + impermeability	1	*E. cloacae*	↑↑↑ Case + CTX-M-15	−	−	−	1.5	6	1
	2	*E. cloacae*	↑↑↑ Case + CTX-M-15	−	−	−	2	8	1
	3	*E. cloacae*	↑↑↑ Case + CTX-M-15	−	+/−	−	3	12	2
	1	*C. freundii*	↑↑↑ Case + TEM-3	−	−	−	1	8	1

Continued

Table 1. *Continued*

	Name	Species	β-Lactamase content[a]	RAPIDEC® CARBA NP[b]	Rapid CARB Screen®[c]	Carba NP test[d]	MIC (mg/L) IPM	ETP	MEM
extended-spectrum	1	*K. pneumoniae*	OXA-163	–	–	–	0.5	0.38	0.12
oxacillinases without	1	*E. cloacae*	OXA-163	–	–	–	0.5	2	0.19
significant carbapenemase activity	1	*S. marcescens*	OXA-405	–	–	–	0.5	0.75	0.19

IPM, imipenem; ETP, ertapenem; MEM, meropenem; CPase, cephalosporinase; ↑↑↑ Case, overexpression of a likely chromosome-encoded cephalosporinase.
[a]Carbapenemase names are in bold.
[b]++, + and +/− are considered positive results and − is considered a negative result.
[c]+ is considered a positive result, +/− is considered an equivocal result (a retest was performed using a higher inoculum as recommended by the manufacturer) and − is considered a negative result.
[d]++ and + are considered positive results, +/− is considered an equivocal result and − is considered a negative result.

(95% CI 93.8%–99.3%)] determined by using a different collection of strains, including Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The performance of the Carba NP test (Table 2) was in accordance with the performance previously described.[6,11–13] As previously reported,[12,14] the Carba NP test performed better than the Rapid CARB Screen®.

Comparison of technical features of the RAPIDEC® CARBA NP, the Rapid CARB Screen® and the Carba NP test

The technical features of the tests are summarized in Table 2. The RAPIDEC® CARBA NP requires only the material included in the kit and a 10 μL inoculation loop. The Rapid CARB Screen® requires additional material/equipment, as does the Carba NP test (Table 2), and a longer time to prepare the reagents.

Although the time required for the three assays is a maximum of 2 h, especially for detection of non-carbapenemase producers, the detection of CPE was obtained faster with the RAPIDEC® CARBA NP and the Carba NP test compared with the Rapid CARB Screen®. Indeed, the median time for the detection of KPC- and MBL-producing isolates was 5 min using RAPIDEC® CARBA NP and the Carba NP test, while it was 30 min for the Rapid CARB Screen® (Table 2). As previously reported,[12] this delay to obtain results by using the Rapid CARB Screen® is mainly due to the time needed to dissolve the tablet containing imipenem plus excipient (tube B) or the excipient alone (tube A) before performing the first reading at 30 min.

The performance of the RAPIDEC® CARBA NP in this study cannot be compared with any other published studies since this study corresponds to the first evaluation of the performance of this test. The performance observed for the Rapid CARB Screen® [sensitivity 89.5% (95% CI 81.7%–94.2%) and specificity 70.9% (95% CI 57.9%–81.2%)] is similar to that previously reported by Huang *et al.*[12] for Enterobacteriaceae (sensitivity 87.1% and specificity 62.7%), confirming the superiority of the Carba NP test compared with the Rapid CARB Screen®. In addition, as noticed by Simner *et al.*[14] and Huang *et al.*,[12] the Rapid CARB Screen® was difficult to interpret compared with the Carba NP test (and now the RAPIDEC® CARBA NP), mostly due to reading difficulties induced by the turbidity of the undissolved tablet containing imipenem plus excipient (tube B) or excipient alone (tube A) (Figure S1). Globally, the performance observed for the Carba NP test [sensitivity 96.8% (95% CI 91.1%–98.9%) and specificity 100% (95% CI 93.5%–100%)] is in line with that reported by Yusuf *et al.*[13] (specificity 91.1% and specificity 100%) and Huang *et al.*[12] (specificity 97% and sensitivity 100%), and with that observed by Pasteran *et al.*[15] for the Blue-Carba test (specificity 97% and sensitivity 100%), a home-made test derived from the Carba NP test.[8] However, the true performance of the Blue-Carba test as compared with the Carba NP test shall be evaluated on an extended panel of carbapenem producers including OXA-48-like producers.

Finally, we showed that the RAPIDEC® CARBA NP is more specific and sensitive than the Rapid CARB Screen® for detecting any type of CPE (known and unknown carbapenemases). It is a rapid and easy-to-handle diagnostic test for controlling the spread of CPE by detecting any kind (known or unknown) of carbapenemase activity. It may find its place as a first-line screen of CPE in clinical settings.

Table 2. Performance and technical features of the RAPIDEC® CARBA NP, the Rapid CARB Screen® and the Carba NP test

	RAPIDEC® CARBA NP			Rapid CARB Screen®			Carba NP test		
Results of the test	negative	positive	equivocal	negative	positive	equivocal	negative	positive	equivocal
non-carbapenemase producers ($n=55$)	55	0	0	39	0	16	55	0	0
carbapenemase producers ($n=95$)	1	94	0	1	85	9	1	92	2
KPC ($n=21$)	0	21	0	0	20	1	0	21	0
NDM ($n=21$)	0	21	0	0	19	2	0	21	0
VIM ($n=17$)	0	17	0	0	17	0	0	17	0
IMP ($n=11$)	0	11	0	0	11	0	0	11	0
OXA-48 ($n=16$)	0	16	0	0	15	1	0	16	0
OXA-48 variants ($n=9$)	1	8	0	1	3	5	1	6	2
Test parameters with equivocal or non-interpretable results considered as invalid results (false positive or false negative)									
sensitivity (%)	99% (95% CI 94.3%–99.8%)			89.5% (95% CI 81.7%–94.2%)			96.8% (95% CI 91.1%–98.9%)		
specificity (%)	100% (95% CI 93.5%–100%)			70.9% (95% CI 57.9%–81.2%)			100% (95% CI 93.5%–100%)		
Time (min) for carbapenemase detection, median (range)									
KPC	5 (5–15)			30			5 (5–15)		
NDM	5 (5–15)			30			5 (5–120)		
VIM	5			30 (30–60)			5 (5–30)		
IMP	5 (5–15)			30			5		
OXA-48	30 (5–60)			30 (30–120)			15 (5–60)		
OXA-48 variants	30 (5–120)			30 (30–120)			30 (15–120)		
Additional material/equipment	10 μL inoculation loop			10 μL inoculation loop Eppendorf tubes physiological serum vortex densitometer			10 μL inoculation loop Eppendorf tubes pH meter vortex weighing balance preparation of the revealing solution imipenem powder		
Minimal time for the first reading	immediate			30 min			immediate		
Endpoint for definitive reading	2 h			2 h			2 h		

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Transparency declarations

An international patent form for the Carba NP test has been filled on behalf of public translational research INSERM Transfert (Paris, France). This patent has been licenced to bioMérieux Inc. and the co-inventors of the patent (P. N., L. P. and L. D.) received fees from INSERM Transfert according to the French law. All other authors: none to declare.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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