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Multicenter evaluation of the RAPIDEC® CARBA NP test for rapid screening of carbapenemase-producing *Enterobacteriaceae* and Gram-negative nonfermenters from clinical specimens

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Abstract

The rapid diagnosis of carbapenemase-producing (CP) bacteria is essential for the management of therapy and infection control. In this study, RAPIDEC® CARBA NP (RCNP) was evaluated for the rapid screening of CP *Enterobacteriaceae, Acinetobacter baumannii* complex and *Pseudomonas aeruginosa* from clinical specimens collected at five Italian hospitals. Firstly, each site tested 20 well-characterized strains in a blinded fashion. Secondly, each center prospectively tested 25 isolates from blood cultures processed with a rapid workflow (6 hours after subculture) and 25 isolates from other specimens processed after an overnight culture. The presence of carbapenemases was confirmed by multiplex Real-Time-PCRs targeting carbapenemase genes. RCNP presented an overall sensitivity, specificity, positive predictive value, and negative predictive value of 70%, 94%, 82%, and 89%, respectively, with a higher performance in detection of CP *Enterobacteriaceae* and a poorer performance in detection of CP *A. baumannii* complex. With isolates from blood cultures, RCNP could significantly reduce the time required for identification of CP *Enterobacteriaceae* (less than 9 hours since the positivization of blood cultures).

1. Introduction

Antibiotic resistance is an issue of growing importance for public health, and involves a large variety of pathogenic bacteria responsible for healthcare-associated and community-acquired infections (Tang et al., 2014). Carbapenems are considered among the last resort antibiotics for treatment of resistant Gram-negatives (Papp-Wallace et al., 2011), but carbapenem-resistant strains of *Enterobacteriaceae* and Gram-negative nonfermenters are now spreading worldwide (Ruppé et al., 2015). The main mechanisms of resistance to carbapenems in Gram-negative pathogens are represented by the production of carbapenemases, reduction of outer membrane permeability mediated by the loss of porin function, and upregulation of efflux systems (Papp-Wallace et al., 2011). The spread of carbapenemase-producing (CP) strains of Gram-negative bacteria (GNB), including *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp., is of notable concern since these strains often carry additional resistance determinants and exhibit complex multidrug-resistant (MDR) phenotypes. Moreover, carbapenemase genes are usually associated with mobile genetic elements and their expression can be associated with higher-level carbapenem resistance (Kaye and Pogue, 2015; Rossolini et al., 2014; Ruppé et al., 2015).

Therefore, rapid identification of CP-GNB is important to implement infection control strategies that limit their spread in hospitals, and to the selection of appropriate antimicrobial therapy (Miriagou et al., 2010). Several approaches can be used for rapid identification of CP-GNB, including phenotypic and genotypic methods (Osei et al, 2015). Among the phenotypic methods, the RAPIDEC® CARBA NP test (bioMérieux, Marcy l'Etoile, France*)* is a commercial test for rapid screening of CP-GNB developed basing on the original CARBA NP colorimetric method (Nordmann et al., 2012). The RAPIDEC® CARBA NP test is easy to use and provides results in two hours, while being cheaper than molecular assays and able to detect also unknown carbapenemase genes. This test, based on the colorimetric detection of hydrolysis of imipenem using phenol red as indicator, has been previously validated or compared with other tests in several studies in which it was retrospectively applied on a collection of isolates previously characterized for the presence of carbapenemases (Poirel and Nordmann, 2015; Dortet et al., 2015; Hombach et al., 2015; Garg et al., 2015; Lifshitz et al., 2016; Kabir et al., 2016; Österblad et al., 2016; Aktaş et al., 2016), or prospectively applied on *Enterobacteriaceae* isolates (Noël et al., 2016).

In this work, we carried out a multicenter evaluation of the RAPIDEC® CARBA NP test, including a proficiency test with well-characterized strains, followed by further testing for the detection of CP-GNB among bacterial isolates prospectively collected from various clinical specimens. Moreover, a fast-track workflow for the detection of CP-GNB using the RAPIDEC® CARBA NP from blood cultures was implemented.

2. Materials and methods

2.1 Participating centers

Five laboratories associated with hospitals located in northern (Lecco and Modena) and central (Florence and Rome) Italy, representative of different Italian Regions, were involved in the study carried out from April to September 2015.

2.2 Proficiency test

A collection of 20 well-characterized strains, previously confirmed as CP (n=14) or carbapenem-resistant but carbapenemase-non-producers (CNP, n=4) or carbapenem-susceptible (n=2) (Table 1), was provided to each participating center in a blinded fashion. Each strain was cultured for 18-24 hours on blood agar and then tested with the RAPIDEC® CARBA NP test according to the Manufacturer's instructions.

2.3 Test on clinical isolates

A total of 250 (50 per participating center) consecutive, non-replicate clinical isolates of *Enterobacteriaceae* and Gram-negative nonfermenters (*P. aeruginosa* and *A. baumannii* complex) were tested with RAPIDEC® CARBA NP. Of them, 125 isolates (25 per participating center) were from blood cultures processed with a fast-track workflow, and 125 isolates (25 per participating center) were from other clinical specimens (surveillance specimens were not included). Positive blood cultures from BACTEC™ (Becton Dickinson, Franklin Lakes, NJ, USA) or BacT/ALERT® (bioMérieux) systems were evaluated with Gram staining and plated onto blood agar plates (bioMérieux). The fast-track workflow foresaw that after six hours of incubation (35±2°C, 5% CO_2), bacterial isolates were identified by MALDI-TOF with the VITEK® MS system (bioMérieux) and, if they belonged to the target species, they were included in the study and tested with the RAPIDEC® CARBA NP test. Since the laboratories did not process positive blood cultures on a 24/7 schedule, only the blood cultures that became positive during the night or in the morning (until 12 a.m.) were processed with the fast-track workflow, by the staff in charge of the afternoon shift. Blood cultures yielding Gram-positive bacteria or mixed Gram-positive/Gram-negative bacteria and/or yeasts at Gram staining were excluded (Figure 1). Urine samples were cultured on chromID® CPS® Elite medium (bioMérieux) for 18-24 hours, while other materials were cultured on blood agar (bioMérieux) for 18-24 hours. Bacterial isolates were identified by MALDI-TOF with the VITEK® MS system (bioMérieux) and, if they belonged to the target species, they were included in the study and tested with the RAPIDEC® CARBA NP (Figure 1).

2.4 RAPIDEC® CARBA NP test

The RAPIDEC® CARBA NP test was performed according to the Manufacturer's instructions, as follows. In case of isolated colonies from 18-24 hour-old cultures, several colonies were deposited in the dedicated well. For the 6-hour bacterial growth from blood cultures, the bacterial growth was transferred directly to the well of RAPIDEC® CARBA NP, until the indicated turbidity was reached. Samples presenting an insufficient bacterial growth were excluded. Strips were incubated at 35±2 °C for up to 120 minutes, and inspected at 30, 60 and 120 minutes. Results were interpreted by comparing the test well and the control well colors. A test was considered positive when a change of color of the well (from red to red-orange, orange or yellow) was observed.

2.5 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed using reference broth microdilution according to CLSI guidelines (CLSI, 2015) and results were interpreted according to EUCAST criteria v 6.0 (http://www.eucast.org/clinical_breakpoints/).

2.6 Molecular detection of carbapenemase genes

After performing the RAPIDEC® CARBA NP, each isolate was processed with three homebrew multiplex Real-Time-PCR mixes for the detection of the main carbapenemase genes, including bla_{KPC}, bla_{VIM}, bla_{NDM}, $bla_{\mathrm{OXA\text{-}48\text{-}like}}$ genes in *Enterobacteriaceae*, bla_{IMP}, bla_{VIM}, $bla_{\mathrm{FIM\text{-}1}}$, bla_{GES} genes in *P. aeruginosa*, and $bla_{\mathrm{OXA\text{-}23\text{-}like}}$, $bla_{\mathrm{OXA\text{-}24\text{-}like}}$, $bla_{\mathrm{OXA\text{-}58\text{-}like}}$, IS*Aba1*+$bla_{\mathrm{OXA\text{-}51\text{-}like}}$ genes for *A. baumannii* complex. Primers and probes used in every reaction, and reaction conditions are described in Table 2. An internal control, consisting of phocine herpesvirus DNA (PhHV), and primers and probe targeting PhHV, was added in each reaction mix as a positive amplification control (Van Doornum et al., 2003).

2.7 Spectrophotometric assay

All meropenem non-susceptible isolates which tested negative with molecular assays for the detection of carbapenemase genes were further investigated by a spectrophotometric assay with crude extracts, using imipenem as substrate, as previously described (Lauretti et al., 1999), for detection of carbapenemase activity to exclude the presence of carbapenemase types not included in the molecular assay.

3. Results

3.1 Proficiency test with RAPIDEC® CARBA NP

Considering that RAPIDEC® CARBA NP is based on a colorimetric method and results are assigned by visual inspection, a proficiency test was initially performed with a collection of 20 well-characterized strains provided to each participating center in a blinded fashion, to evaluate the reproducibility of interpretation of results obtained at different centers.

All the CNP (n=4) and the susceptible (n=2) strains were correctly identified as carbapenemase-negative with the RAPIDEC® CARBA NP test by all laboratories, while four of the 14 CP strains were not reported as carbapenemase-positive by one or more laboratories. In particular, the OXA-58-positive *A. baumannii* complex strain was not detected as CP by two laboratories, while the FIM-1-positive and the IMP-13-positive *P. aeruginosa* strains, and the OXA-372-positive *Citrobacter freundii* strain, were not detected as CP by one laboratory each (Table 1).

The overall good results of the proficiency test (specificity for detection of CP, 100%; sensitivity for detection of CP, 93%) prompted us to proceed with the analysis of isolates from clinical samples in each center.

3.2 Performance of the RAPIDEC® CARBA NP test on isolates from clinical specimens other than blood cultures

Among 125 isolates from various materials (mainly urine and respiratory specimens), 31 were confirmed as CP by molecular methods. These isolates included 10 KPC-producing *K. pneumoniae*, one OXA-48-producing *K. pneumoniae*, one KPC- and VIM-coproducing *K. pneumoniae,* and 19 class D carbapenemase-producing *A. baumannii* complex (n=16 OXA-23-like, n=2 OXA-24-like, n=1 OXA-51-like overexpressed by an IS*Aba1* inserted upstream). Of these, the RAPIDEC® CARBA NP test correctly detected the 12 CP *Enterobacteriaceae*, but failed to identify 11 of the 19 CP *A. baumannii* complex (including 9 positive for a $bla_{\text{OXA-23-like}}$ gene, one positive for a $bla_{\text{OXA-24-like}}$ gene, and one carrying a $bla_{\text{OXA-51-like}}$ gene preceded by an IS*Aba1* insertion sequence). Moreover, the RAPIDEC® CARBA NP test was positive with one *K. pneumoniae* and five *P. aeruginosa* for which the molecular tests and the spectrophotometric assay had not identified carbapenemases (Table 3). Consequently, overall sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPP) were found to be 65%, 94%, 77%, and 89%, respectively. If *A. baumannii* complex strains were not included in the study, the sensitivity, specificity, PPV and NPP of RAPIDEC® CARBA NP would be 100%, 93%, 67%, 100%, respectively. Considering only the 61 *Enterobacteriaceae* isolates, the test yielded an even better performance (100%, 98%, 92%, 100%, respectively) (Table 4).

3.3 *Performance of the RAPIDEC® CARBA NP test with positive blood cultures in a fast-track workflow*

Of the 125 isolates from blood cultures tested with RAPIDEC® CARBA NP, 122 gave interpretable results, while three isolates (one *P. aeruginosa*, one *A. baumannii* complex and one *Enterobacter cloacae*) yielded insufficient growth to perform the test at 6 hours after subculture. Among the 122 evaluable isolates, 36 were confirmed as CP by molecular methods, including 18 KPC-producing *K. pneumoniae*, two OXA-48-producing *K. pneumoniae*, one VIM-producing *K. pneumoniae,* three VIM-producing *P. aeruginosa* and 12 class D carbapenemase-producing *A. baumannii* complex (n=10, OXA-23-like; n=1, OXA-24-like; n=1, OXA-23-like and OXA-24-like co-producer). Of them, RAPIDEC® CARBA NP assay correctly identified 27 isolates as CP, but missed nine *A. baumannii* complex carrying $bla_{\text{OXA-23-like}}$ and/or $bla_{\text{OXA-24-like}}$ genes (Table 3). RAPIDEC® CARBA NP correctly categorized as CNP 82 out of 86 isolates from blood cultures, while false-positive results were obtained with one *K. pneumoniae* and three *P. aeruginosa* isolates for which no carbapenemase genes nor carbapenemase activity were detected using Real-Time-PCRs and spectrophotometric activity, respectively. According to these results, the RAPIDEC® CARBA NP showed a sensitivity of 75%, a specificity of 95%, a PPV of 87%, and a NPV of 90%. However, excluding the *A. baumannii* complex isolates, sensitivity, specificity, PPV and NPV were 100%, 95%, 86%, 100%, respectively, and these percentages further increased when considering only the 81 *Enterobacteriaceae* isolates (100%, 98%, 95%, 100%, respectively) (Table 4).

3.4 *Overall performance of the RAPIDEC® CARBA NP test with clinical isolates*

Overall, considering all types of clinical specimens, the RAPIDEC® CARBA NP test yielded sensitivity, specificity, PPV and NPP of 70%, 94%, 82%, and 89%, respectively. The best results were observed with *Enterobacteriaceae*, as described in Table 4.

No significant differences were observed among the performance of the RAPIDEC® CARBA NP test carried out at the five centers.

4. Discussion

The global spread of CP-GNB represents a major public health challenge. Clinical Microbiology laboratories are increasingly asked for rapid detection of CP strains for infection control and antimicrobial stewardship purposes.

The RAPIDEC® CARBA NP test is a simple phenotypic test that does not require any specific technical skills or expensive equipment (Poirel and Nordmann, 2015; Garg et al., 2015), which allows rapid detection of CP strains in a timeframe comparable to that of molecular tests (i. e. 1-2 hrs) but at a substantially lower cost.

In this multicenter work, involving five different Italian laboratories, we evaluated the performance of RAPIDEC® CARBA NP in a proficiency test carried out by each laboratory with a collection of 20 well-characterized carbapenem-resistant strains representative of different Gram-negative species and resistance mechanisms, and then in a field test with 50 clinical isolates from each laboratory.

Results revealed an overall high specificity of RAPIDEC® CARBA NP for detection of CP strains, similar to that previously reported by Poirel and Nordmann (2015) (96%), Garg et al. (2015) (96.2%) and Kabir et al. (2016) (98.5%). The false-positive results, observed with *K. pneumoniae* and *P. aeruginosa* isolates, and reported also by other Authors (Poirel and Nordmann, 2015; Lifshitz et al., 2016; Österblad et al., 2016), could be attributable to a reduced stability of the imipenem substrate used in the test toward strains producing enzymes that are not true carbapenemases but have some weak carbapenemase activity (e. g. AmpCs, CMY, CTX-M-type producers), and possibly also to the use of the inoculum recommended in the Manufacturer's instructions. Indeed, Dortet et al. (2015) identified a critical impact of the bacterial inoculum in the performance of the test, and recommended the use of a much higher inoculum to avoid false positive results.

On the other hand, the test frequently failed to detect *A. baumannii* complex strains producing class D β-lactamases, a problem reported also by other authors (Poirel and Nordmann, 2015; Kabir et al., 2016) and probably due to the overall weak carbapenemase activity of class D carbapenemases (Queenan and Bush, 2007). A lower overall sensitivity (70%) was detected in this work compared to previously reported studies by Garg et al. (2015) (92.6%), Kabir et al. (2016) (97.8%), and Poirel and Nordmann (2015) (96%). This difference could be partially ascribed to the higher percentage of class D producing *A. baumannii* complex (12.5%) tested in our study (Garg et al. [2015] 0%, Kabir et al. [2016] 4.7%, and Poirel and Nordmann [2015] 8.5%). Also Noël et al. (2016) showed that RAPIDEC® CARBA NP performed poorly for the detection of class D carbapenemase-producing *Acinetobacter* spp. isolates (sensitivity 36.4%, specificity 75%), leading us to conclude that this test should not be used with *A. baumannii* complex isolates in its present format. A much higher bacterial inoculum, compared to the Manufacturer's instructions, could increase the sensitivity of the tests (Lifshitz et al., 2016). Indeed, Dortet et al. (2015) recommended to perform the test using a standardized inoculum (a full 10 μl loop), which is critical for test reliability.

A limitation of the prospective evaluation of RAPIDEC® CARBA NP carried out in this work is represented by the relatively low number of isolates producing some types of carbapenemases (e. g. OXA-48 or VIM), and of carbapenemase-producing *P. aeruginosa* isolates, which reflected the local epidemiology of infections.

Considering *Enterobacteriaceae* isolates only, the values of sensitivity and specificity were high and comparable with other works (Dortet et al., 2015; Hombach et al., 2015; Lifshitz et al., 2016), showing that RAPIDEC® CARBA NP test could be useful also for detecting CP *Enterobacteriaceae* from positive blood cultures processed with a fast-workflow approach. With this approach, RAPIDEC® CARBA NP can be used to reduce the time required for identification of CP *Enterobacteriaceae* to less than 9 hours since positivization of blood cultures (from at least 24 to 48 hours of routine methods) (Morgenthaler and Kostrzewa, 2015). The rapid identification of CP Gram-negatives from blood cultures can be of remarkable importance to antimicrobial stewardship (Barlem et al., 2016). It should be noted, however, that the possibility of using a fast workflow is dependent on the laboratory schedule. For instance, in the laboratories participating in this work, which do not process positive blood cultures on a 24/7 schedule, the fast workflow could only be performed with blood cultures that were found to be positive or became positive in the morning. When the RAPIDEC® CARBA NP is used with positive blood cultures in the rapid workflow, another limitation could be represented by an insufficient bacterial growth for inoculum at 6 hours. However, in our experience this occurred only with a small number of cases (3 of 122, 2.4%).

A limitation of the RAPIDEC® CARBA NP test is that it cannot discriminate the type of carbapenemase produced. This information is important for antimicrobial stewardship since the new antibiotics active against CP-GNB that are entering clinical practice (e. g ceftazidime-avibactam) may not cover all types of CP strains. In this perspective, the RAPIDEC® CARBA NP could have a role as a screening test for excluding carbapenemase production and selecting the most suitable candidates for characterization of the carbapenemase type by molecular platforms or immunoenzymatic assays (Banerjee et al., 2015; Raich and Powell, 2015; Meunier et al., 2016).

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Figure 1. Workflow for the detection of carbapenemase producers in different clinical isolates. Significant pathogen indicates the presence of a suspect pathogen in a significant count (if applicable for the specimen type), present as a pure culture or as a mixed population with commensals derived from sampling the nonsterile site, considered to be clinically significant and subjected to further identification, AST and reporting.

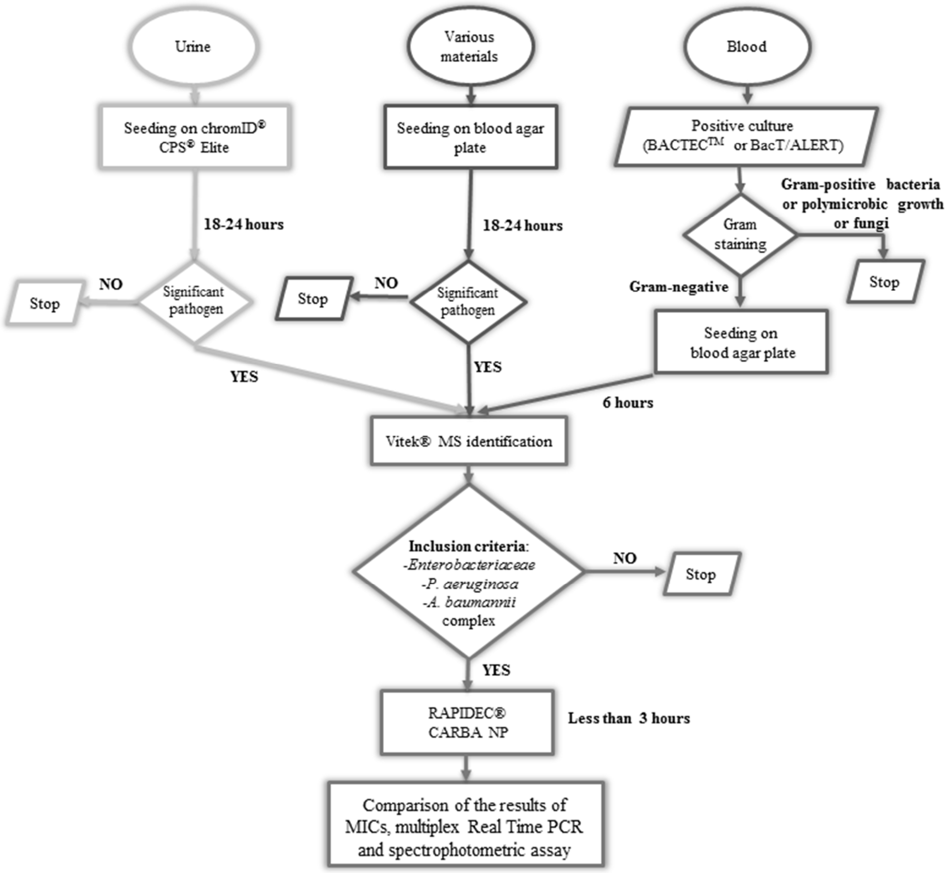

Table 1
Gram-negative strains selected for the evaluation of RAPIDEC® CARBA NP proficiency with the test results obtained in each site.

Strain	Species	Principal Acquired β-lactamase	Reference	MIC Meropenem (μg/mL)	RAPIDEC® results (center)					
					Expected	1	2	3	4	5
6-419	*Escherichia coli*	No-one	-	0.5	–	–	–	–	–	–
23-1786	*Enterobacter ludwigii*	NMC-A	Antonelli et al., 2015c	32	+	+	+	+	+	+
7-556	*K. pneumoniae*	NDM-1	-	32	+	+	+	+	+	+
22-1706	*E. coli*	NDM-5	-	>32	+	+	+	+	+	+
7728	*P. aeruginosa*	IMP-13	-	4	+	+	–	+*	+	+
ATCC 25922	*E. coli*	no-one	-	0.5	–	–	–	–	–	–
47-3752	*E. cloacae* complex	IMI-2	-	>32	+	+	+	+	+*	+
CVB-1	*E. coli*	NDM-1	D'Andrea et al., 2011	32	+	+	+	+	+	+
ECBZ-1	*E. coli*	OXA-48	Giani et al., 2012	1	+	+	+	+	+*	+
FIPP-1	*K. pneumoniae*	KPC-3	Giani et al., 2009	>32	+	+	+	+	+	+
VA-417/02	*E. cloacae* complex	VIM-4	Luzzaro et al., 2004	32	+	+	+	+	+	+
FI-14/157	*P. aeruginosa*	FIM-1	Pollini et al., 2013	>32	+	+	–	+	+	+
Cfr-FI-07	*C. freundii*	OXA-372	Antonelli et al., 2015b	16	+	+	+	+	–	+
45A02	*K. pneumoniae*	FOX-7 + porin deficiency	Arena et al., 2013	4	–	–	–	–	–	–
NV132	*A. baumannii* complex	OXA-58	-	8	+	+*	–	+	+*	–
8-27	*K. pneumoniae*	CTX-M-1-like + OMP deficient	-	2	–	–	–	–	–	–
10-52	*K. pneumoniae*	CTX-M-1-like + OMP deficient	-	2	–	–	–	–	–	–
VA-416/02	*K. pneumoniae*	VIM-4	Luzzaro et al., 2004	32	+	+	+	+	+	+
GW1	*P. aeruginosa*	GES-2	Poirel et al., 2001	16	–	–	–	–	–	–
PIEcl	*E. cloacae* complex	VIM-1	-	>32	+	+	+	+	+	+

* Borderline positive: there was correctly a change of color in the test well, but it was not clear as described in manufacturer's instructions.

Table 2

Primers and probes used in this study for three different multiplex Real Time PCR.

Investigated bacteria	Target	Primer name	Sequence (5'-3')	Reference	Positive control	Concentration of use in reaction mix (nM)
Enterobacteriaceae [a]	*bla*$_{OXA-48-like}$ genes	OXA-48-like-rt-F	GTAGCAAAGGAATGGCAAGAAA	Antonelli et al., 2015a	*E. coli* ECBZ-1 (OXA-48) (Giani et al., 2012)	500
		OXA-48-like-rt-R	GATGCGGGTAAAAATGCTTG	Antonelli et al., 2015a		500
		OXA-48-like-rt-P	HEX-CTCTGGAATGAGAATAAGCAGCAAGG-BHQ-1	Antonelli et al., 2015a		125
	bla$_{KPC}$ genes	kpc-hind-fwd	GATACCACGTTCCGTCTGG	Hindiyeh et al., 2008	*K. pneumoniae* FIPP-1 (KPC-3) (Giani et al., 2009)	500
		kpc-hind-rev	GCAGGTTCCGGTTTTGTCTC	Hindiyeh et al., 2008		500
		kpc-hind-tq	FAM-AGCGGCAGCAGTTTGTTGATTG-BHQ-1	Hindiyeh et al., 2008		125
	bla$_{VIM}$ genes [b]	VIM-rt-fwd	TGGTCTCATTGTCCGTGATG	Antonelli et al., 2016	*K. pneumoniae* VA-416/02 (VIM-4), (Luzzaro et al., 2004)	500
		VIM-rt-rev	CATGAAAGTGCGTGGAGA	Antonelli et al., 2016		500
		VIM-rt-tq	ROX-AAGCAAATTGGACTTCCCGTAACGC-BHQ-2	Antonelli et al., 2016		125
	bla$_{NDM}$ genes	*bla*NDM1_F	CGCAACACAGCCTGACTTT	Ong et al., 2008	*E. coli* CVB-1 (NDM-1) (D'Andrea et al., 2011)	500
		*bla*NDM1_R	TCGATCCCAACGGTGATATT	Ong et al., 2008		500
		*bla*NDM1_P	CY5-CAACTTTGGCCCGCTCAAGGTATTT-BHQ-3	Ong et al., 2008		125
P. aeruginosa [c]	*bla*$_{FIM-1}$ gene	FIM-rt-F	CGCCTTAACACCCGTCGTGA	This study	*P. aeruginosa* FI-14/157 (FIM-1) (Pollini et al., 2013)	500
		FIM-rt-R	GTCTCCTTTTTCAACGATTAGCC	This study		500
		FIM-rt-P	HEX- CTGGCGTACAAGCGGCTCAACCCAA- BHQ-1	This study		125
	bla$_{GES}$ genes	GES-rt-F	AGAATTGACTCAGGCACCGAG	This study	*P. aeruginosa* GW1 (GES-2) (Poirel et al., 2001)	500
		GES-rt-R	GTTAGTAGCCCCATTGTCGC	This study		500
		GES-rt-P	CY5- GAACCGTCATGTGTCCCGATGCTAG-BHQ-3	This study		125
	bla$_{IMP}$ genes	IMP-rt-F	GANGCYTAYHTRATWGAYACTCCA	This study	*P. aeruginosa* FI-5/7728 (IMP-13), unpublished	2000
		IMP-rt-R	GRRATDGAYYGAGARTTAAGCCA	This study		2000
		IMP-rt-P	FAM- ATTCCNSCYGHRCTRTCRCYATGRAAATG- BHQ-1	This study		250
A. baumannii complex [c]	*bla*$_{OXA-23-like}$ genes	oxa-23-like-rt-F	GATTGTTCAAGGACATAATCAGGTG	This study	*A. baumannii* Ab13 (OXA-23) (Coverc et al., 2007)	500
		oxa-23-like-rt-R	GGTTCTCCAATCCGATCAGGG	This study		500
		oxa-23-like-rt-P	FAM- AGGCTGGCACATATTCTGTATTTGCGG- BHQ-3	This study		125
	bla$_{OXA-24-like}$ genes	oxa-24-like-rt-F	CTTCCTATHYTCAGCATTTCTATTCTAG	This study	*A. baumannii* VA-566/00 (OXA-40/24), (D'Andrea et al., 2009)	1000
		oxa-24-like-rt-R	ATCTTAAATGTTGAYGCAGGGAC	This study		*1000*
		oxa-24-like-rt-P	HEX- GCTATTTTGATGAAGCTCAAACACARGGT- BHQ-1	This study		250
	bla$_{OXA-58-like}$ genes	oxa-58-like-rt-F	AAAGCATGGGACAAAGATTTTAC	This study	*A. baumannii NV132* (OXA-58), unpublished	500
		oxa-58-like-rt-R	CAAACTTTACTTCTTGTATAGGTGT	This study		500
		oxa-58-like-rt-P	ROX- CAGTGCCTGTATATCAAGAATTGGCAC- BHQ-2	This study		125
	IS*Aba1*+ *bla*$_{OXA-51-like}$ genes	ISABA1-oxa-51-rt-F	ATAATCACAAGCATGATGAGCG	This study	*A. baumannii 696/03* (OXA-58), unpublished	500
		ISABA1-oxa-51-rt-R	GTGARCAGGCTGAAATARRAATAG	This study		500
		ISABA1-oxa-51-rt-P	CY5- ATGAACATTAAAGCACTCTTACTTATAACAAG- BHQ-3	This study		125
Everyone	PhHV (internal control)	PhHV-267s	GGG CGA ATC ACA GAT TGA ATC	Van Doornum et al., 2003	PhHV DNA cloned in pGEM-T-easy *E. coli* DH5α	500
		PhHV-337as	GCG GTT CCA AAC GTA CCA A	Van Doornum et al., 2003		500
		PhHV-305tq	Cy5.5 -TTTTATGTGTCCGCCACCATCTGGATC-BHQ-3	Van Doornum et al., 2003		125

[a]The amplification program consisted of 35 two-step cycles of 15 s at 95°C and 60 s at 60°C.

[b]*bla*$_{VIM}$ genes were also target of the multiplex Real Time PCR used for *P. aeruginosa* isolates.

[c]The amplification program consisted of 35 three-step cycles of 15 s at 95°C, 30 s at 50°C and 30 s at 60°C.

Table 3
Comparison of the results obtained from RAPIDEC® CARBA NP test with the molecular detection of carbapenemase genes. Discrepant RAPIDEC® CARBA NP results are underlined.

Origin of Isolates	Species	No. of isolates	MIC MEM (μg/ml)	Carbapenemase genes	RAPIDEC® Result
various materials	*Enterobacteriaceae*	10	16 to >128	bla_{KPC}	+
		1	32	$bla_{OXA\text{-}48\text{-}like}$	+
		1	16	bla_{KPC}+bla_{VIM}	+
		48	≤0.03 to 4	No one	–
		1	0.25	No one	+
	P. aeruginosa	4	16 to >128	No one	+
		35	≤0.03 to 64	No one	–
		1	2	No one	+
	A. baumannii complex	7	64 to >128	$bla_{OXA\text{-}23\text{-}like}$	+
		9	8 to 128	$bla_{OXA\text{-}23\text{-}like}$	–
		1	64	$bla_{OXA\text{-}24\text{-}like}$	+
		1	2	$bla_{OXA\text{-}24\text{-}like}$	–
		1	2	IS*Aba1*+$bla_{OXA\text{-}51\text{-}like}$	–
		5	0.25 to 8	No one	–
blood cultures	*Enterobacteriaceae*	18	8 to >128	bla_{KPC}	+
		2	1 to 4	$bla_{OXA\text{-}48\text{-}like}$	+
		1	4	bla_{VIM}	+
		59	≤0.03 to 2	No one	–
		1	≤0.03	No one	+
		1	≤0.03	No one	[a]
	P. aeruginosa	3	8 to >128	bla_{VIM}	+
		1	>128	bla_{VIM}	[a]
		1	>128	No one	+
		20	0.12 to >128	No one	–
		2	0.12; 0.5	No one	+
	A. baumannii complex	1	128	$bla_{OXA\text{-}23\text{-}like}$ and $bla_{OXA\text{-}24\text{-}like}$	–
		7	16 to >128	$bla_{OXA\text{-}23\text{-}like}$	–
		3	16 to 64	$bla_{OXA\text{-}23\text{-}like}$	+
		1	>128	$bla_{OXA\text{-}24\text{-}like}$	–
		1	>128	$bla_{OXA\text{-}23\text{-}like}$	[a]
		3	0.25	No one	–

[a] insufficient biomass 6 hours after subculture.

Table 4
Performance of RAPIDEC® CARBA NP test with clinical isolates.

Sources	**Species/Family**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
All	**All (N=247)**	**70**	**94**	**82**	**89**
	Excluding *A. baumannii* complex (N=208)	100	94	78	100
	Enterobacteriaceae (N=142)	100	98	94	100
Blood cultures	**All (N=122)**	**75**	**95**	**87**	**90**
	Excluding *A. baumannii* complex (N=107)	100	95	86	100
	Enterobacteriaceae (N=81)	100	98	95	100
Other materials	**All (N=125)**	**65**	**94**	**77**	**89**
	Excluding *A. baumannii* complex (N=101)	100	93	67	100
	Enterobacteriaceae (N=61)	100	98	92	100

Highlights

- RAPIDEC® CARBA NP could detect carbapenemase producers from positive blood cultures, after 6 hours of subculture
- The test showed low sensitivity with carbapenemase-producing *A. baumannii*
- Excluding *A. baumannii* the test showed high sensitivity (100%) and specificity (94%)