Diagnostic Microbiology and Infectious Disease 88 (2017) 207–213

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Bacteriology

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

Marco Coppi [a], Alberto Antonelli [a], Tommaso Giani [b], Teresa Spanu [c], Flora Marzia Liotti [c], Carla Fontana [d,e], Walter Mirandola [e], Raffaele Gargiulo [f], Agostino Barozzi [f], Carola Mauri [g], Luigi Principe [g], Gian Maria Rossolini [a,h,*]

[a] Department of Experimental and Clinical Medicine, University of Florence, Italy
[b] Department of Medical Biotechnologies, University of Siena, Siena, Italy
[c] Institute of Microbiology, Catholic University of the Sacred Heart, Agostino Gemelli Hospital, Rome, Italy
[d] Department of Experimental Medicine and Surgery, Tor Vergata University of Rome, Rome, Italy
[e] Clinical Microbiology Laboratories, Polyclinic of Tor Vergata Foundation, Rome, Italy
[f] Provincial Laboratory of Clinical Microbiology, S. Agostino-Estense Hospital, Modena, Italy
[g] Microbiology and Virology Unit, A. Manzoni Hospital, Lecco, Italy
[h] Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy

ARTICLE INFO

Article history:
Received 17 January 2017
Received in revised form 18 April 2017
Accepted 20 April 2017
Available online 27 April 2017

Keywords:
CPE
Blood culture
Fast-track workflow
Rapid screening

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 h after subculture) and 25 isolates from other specimens processed after an overnight culture. The presence of carbapenemases was confirmed by multiplex real-timePCRs 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 h 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 up-regulation 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 Sekyere 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 2 h, while being cheaper than

* Corresponding author. Tel.: +39-0557949239.
E-mail address: gianmaria.rossolini@unifi.it (G.M. Rossolini).

http://dx.doi.org/10.1016/j.diagmicrobio.2017.04.009
0732-8893/

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 (Aktaş et al., 2016; Dortet et al., 2015; Garg et al., 2015; Hombach et al., 2015; Kabir et al., 2016; Lifshitz et al., 2016; Österblad et al., 2016; Poirel and Nordmann, 2015), or prospectively applied on *Enterobacteriaceae* isolates (Noël et al., 2017).

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 h 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 6 h 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 (Fig. 1). Urine samples were cultured on chromID® CPS® Elite medium (bioMérieux) for 18–24 h, while other materials were cultured on blood agar (bioMérieux) for 18–24 h. 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 (Fig. 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 to 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)

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.