Diagnostic Microbiology and Infectious Disease 99 (2021) 115280

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

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

Original Article

Rapid detection of carbapenemase-producing *Pseudomonas* spp. using the NitroSpeed-Carba NP test

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ARTICLE INFO

Article history:
Received 6 October 2020
Revised in revised form 21 November 2020
Accepted 21 November 2020
Available online 27 November 2020

Keywords:
Carbapenemase
Pseudomonas
Detection
Biochemistry

ABSTRACT

The NitroSpeed-Carba NP test was used to rapidly detect and discriminate between the different types of carbapenemases (classes A, B, and D) within 30 minutes among a collection of 202 *Pseudomonas* sp. strains (mostly *Pseudomonas aeruginosa*). A total of 99 carbapenemase−(including enzymes exhibiting weak carbapenemase activity such as several Guyana Extended-Spectrum (GES)-ß-lactamases) and 103 non-carbapenemase producers were tested, and the overall specificity and sensitivity were 100% and 99%, respectively. The NitroSpeed-Carba NP test is a rapid, specific, sensitive, and easy-to-implement technique for identification of carbapenemase-producing *Pseudomonas* spp.

Pseudomonas aeruginosa is one of the most frequent human pathogen, in particular as a source of acquired pneumonia in intensive care units with a high proportion of those strains being multidrug resistant (Boucher et al., 2009; Fernández-Barat et al., 2017). Carbapenem-resistant *Pseudomonas* spp. are increasingly reported in health care facilities (Nordmann and Poirel, 2019; Shin and Park, 2017; Vincent et al., 2009; Wright et al., 2017). Carbapenem resistance in *Pseudomonas* spp. may be related to different mechanisms, such as a decreased outer membrane permeability, with or without overexpression of the intrinsic AmpC-type cephalosporinase, or acquisition of carbapenemases (Mesaros et al., 2007; Rodríguez-Martínez et al., 2009). The most commonly reported carbapenemases in *Pseudomonas* spp. are metallo-β-lactamases (e.g., Verona Imipenemase (VIM), Imipenemase (IMP), Sao Paulo metallo enzyme (SPM), German imipenemase (GIM), and New Dehli metallo b-lactamase (NDM) types) and, to a lesser extent, Ambler class A carbapenemases (e.g., Klebsiella pneumoniae carbapenemase (KPC) and some Guyana Extended-Spectrum (GES)-type enzymes; Gupta, 2008; Jovcic et al., 2011; Mesaros et al., 2007; Poirel et al., 2010; Rodríguez-Martínez et al., 2009; Sadek et al., 2020; Strateva and Yordanov, 2009; Voor et al., 2014). There are also extremely rare reports of *P. aeruginosa* isolates producing carbapenem-hydrolyzing class D ß-lactamases such as OXA-181 (Meunier et al., 2016). Therefore, a rapid, easy, accurate and cost-effective detection of carbapenemase producers is one of the priorities in clinical microbiology in order to assist the clinical decision-making for treating infected patients and for the implementation of infection control measures.

Very recently, a biochemical test (NitroSpeed-Carba NP test) was developed to identify carbapenemase production in Enterobacterales and to discriminate between the different types of clinically-significant carbapenemases (Nordmann et al., 2020). The NitroSpeed-Carba NP test is based on the hydrolysis of the nitrocefin substrate by any ß-lactamase including all carbapenemases, and the capacity of ertapenem to prevent this hydrolysis from all ß-lactamases except from carbapenemases. Specific carbapenemase inhibitors of class A (avibactam, vaborbactam), class B (dipicolinic acid), and class D (avibactam) were used to allow the identification of the carbapenemase types. The aim of the present study was to evaluate the NitroSpeed-Carba NP test to rapidly detect carbapenemases and discriminate between the different types of carbapenemases (classes A, B, and D) in *Pseudomonas* spp.

A well-characterized panel of 202 clinical isolates belonging to different *Pseudomonas* species, mostly *P. aeruginosa*, obtained from various clinical samples and from various countries, have been included in the study (Tables 1 and 2). All strains had previously been characterized for their ß-lactamase content and carbapenem resistance mechanisms by PCR approaches followed by subsequent DNA sequencing of the corresponding amplicons. The isolates included 99 carbapenemase producers and 103 non-carbapenemase producers. The carbapenemase types were as follows: class A carbapenemases n = 8 (KPC, GES, Bicêtre Carbapenemae (BIC)), class B carbapenemases n = 90 (VIM, NDM, IMP, GIM, DIM, SPM, AIM), class D

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https://doi.org/10.1016/j.diagmicrobio.2020.115280

Table 1
Features of carbapenemase-producing *Pseudomonas* sp. isolates, MICs of carbapenems and results of the NitroSpeed-Carba NP test.

Ambler class (n)	Carbapenemase type	Organism	β-Lactamase (n)	MIC range (mg/L)		NitroSpeed-Carba NP result				
				IPM	MEM	Water	ETP	ETP+DPA	ETP+AVI	ETP+VAB
Class A (8)	KPC	*P. aeruginosa*	KPC-2 (4)	>32	>32	+	+	+	–	–
	GES	*P. aeruginosa*	GES-2 (1)	3	1	+	–	–	–	–
		P. aeruginosa	GES-5 (2)	>32	>32	+	+	+	–	–
	BIC	*P. fluorescens*	BIC-1 (1)	>32	4	+	+	+	–	–
Class B (90)	VIM	*P. aeruginosa*	VIM-1 (8)	>32	>32	+	+	–	+	+
		P. aeruginosa	VIM-2 (30)	>32	>32	+	+	–	+	+
		P. stutzeri	VIM-2 (1)	>32	>32	+	+	–	+	+
		P. putida	VIM-2 (2)	>32	>32	+	+	–	+	+
		P. fluorescens	VIM-2 (1)	>32	>32	+	+	–	+	+
		P. aeruginosa	VIM-4 (10)	>32	>32	+	+	–	+	+
		P. putida	VIM-5 (1)	>32	>32	+	+	–	+	+
		P. aeruginosa	VIM-6 (1)	>32	>32	+	+	–	+	+
		P. aeruginosa	VIM-53 (3)	>32	>32	+	+	–	+	+
	IMP	*P. aeruginosa*	IMP-1 (4)	12–>32	>32	+	+	–	+	+
		P. stutzeri	IMP-1 (1)	2	4	+	+	–	+	+
		P. putida	IMP-1 (1)	1	0.19	+	+	–	+	+
		P. aeruginosa	IMP-4 (1)	>32	>32	+	+	–	+	+
		P. aeruginosa	IMP-7 (2)	>32	>32	+	+	–	+	+
		P. aeruginosa	IMP-10 (1)	>32	>32	+	+	–	+	+
		P. aeruginosa	IMP-13 (3)	>32	>32	+	+	–	+	+
		P. aeruginosa	IMP-15 (1)	>32	>32	+	+	–	+	+
		P. aeruginosa	IMP-19 (1)	>32	>32	+	+	–	+	+
		P. aeruginosa	IMP-29 (1)	>32	>32	+	+	–	+	+
	NDM	*P. aeruginosa*	NDM-1 (8)	>32	>32	+	+	–	+	+
	AIM	*P. aeruginosa*	AIM-1 (2)	>32	>32	+	+	–	+	+
	GIM	*P. aeruginosa*	GIM-1 (2)	>32	>32	+	+	–	+	+
	DIM	*P. stutzeri*	DIM-1 (1)	>32	>32	+	+	–	+	+
	SPM	*P. aeruginosa*	SPM-1 (4)	>32	>32	+	+	–	+	+
Class D (1)	OXA-48	*P. aeruginosa*	OXA-181 (1)	32	>32	+	+	+	–	+

MIC = Minimum Inhibition Concentration; IPM = Imipenem; MEM = meropenem; DPA = dipiclonic acid; AVI = avibactam; VAB = Vaborbactam; **+**, change of solutin color from yellow to red; **-**, no change of solution color remaining yellow.

carbapenemase n = 1 (OXA-181) (Table 1). The 103 non-carbapenemase producing-*Pseudomonas* strains were representative of the main β-lactam resistance phenotypes and β-lactamase diversity identified in *Pseudomonas* spp., among which 51% (53/103) were imipenem nonsusceptible, and 20% (21/103) were additionally meropenem nonsusceptible (Table 2). Minimal inhibitory concentrations of meropenem and imipenem was determined using Etest strips (bioMérieux, La Balme-les-Grottes, France) on Mueller-Hinton agar plates at 37 °C, and the results were interpreted according to the latest EUCAST breakpoints (www.eucast.org).

The same protocol of the NitroCarba-Carba NP test as the one used for Enterobacterales was followed (Nordmann et al., 2020). A 1 to 2 calibrated 1 μL-loopful of the strain to be tested was resuspended in 5 different tubes, namely containing 100 μL of a Tris-HCl 20 mmol/L lysis buffer (B-PERII, Bacterial Protein Extraction Reagent; Thermo Scientific Pierce, Rockford, IL, USA) and 0.1 mM $ZnSO_4$ (tubes 1 and 2), either supplemented with 25 mM dipicolinic acid (tube 3), or supplemented with 0.04 mM avibactam sodium hydrate (tube 4), or supplemented with 50 μg/mL vaborbactam (tube 5). After vigorous vortexing, distilled water was added to tube 1 (control) while 50 μL of ertapenem sodium (ETP, 80 μg/mL) was added to tubes 2 to 5. Following incubation at room temperature for 5 minutes, 50 μL of 1 g/L nitrocefin was added into all tubes. The final concentrations of ETP were therefore corresponding to 20 μg/mL.

Interpretation of the test is as follows; a color change from yellow to red (indicating hydrolysis of nitrocefin) in tubes 1, 2, and 3 and its absence in tubes 4 and 5 indicated production of a class A carbapenemase. A color change in tubes 1, 2, 4, and 5 and its absence indicated production of a class B carbapenemase. A color change in tubes 1, 2, 3, and 5 and the absence of any color change in tube 4 indicated production of a class D carbapenemase. A color change in tube 1 only and the absence of any color change in the other tubes indicated lack of carbapenemase activity but production of a non-carbapenemase ß-lactamase. Finally, absence of any color change in all tubes indicates lack of β-lactamase activity. All results are interpreted within 15 minutes (0–15 minutes) of incubation at room temperature.

For the detection of carbapenemase production in *Pseudomonas* sp., the overall specificity of the NitroSpeed-Carba NP test was 100%. All carbapenemase-producing isolates hydrolyzed nitrocefin in the ertapenem-free tube 1 and in tube 2 containing ertapenem, with the exception of a single GES-2 type producer (Table 1). However, GES-5 carbapenemase producers were well detected. GES-5 is known to exhibit a higher carbapenemase activity than GES-2, that latter possessing a very weak carbapenemase activity (Kotsakis et al., 2010). The NitroSpeed-Carba NP test efficiently differentiated carbapenemase producers from non-carbapenemase producers. Hence, isolates being carbapenem-resistant through different combined mechanisms of resistance (outer membrane permeability defect associated or not with overproduction of an AmpC- or extended-spectrum β-lactamase [ESBL]-type enzymes) all gave negative results. Likewise, negative results were obtained with those isolates overproducing their AmpC or being ESBL (lacking carbapenemase activity) producers and remaining carbapenem susceptible (Table 2).

The overall sensitivity of the NitroSpeed-Carba NP test for detecting class A and class B carbapenemases was 99%. Sensitivity of detection of class D carbapenemases could not be calculated but a positive result was obtained for the single OXA-181 producer tested, although this positivity was obtained more lately (30 minutes). However, the specificity of detection of class D carbapenemases was excellent with no carbapenemase-negative producer being falsely detected as positive, which is crucial owing the high occurrence of carbapenem nonhydrolyzing OXA-type enzymes in *P. aeruginosa* (particularly OXA-10 derivatives). Noteworthy, the NitroSpeed-Carba NP test also

Table 2
Features of non-carbapenemase producing-*Pseudomonas* isolates, MICs of carbapenems and results of the NitroSpeed-Carba NP test.

Resistance mechanism (s)	Organism (n)	Resistance determinant(s)	MIC range (mg/L)		NitroSpeed-Carba NP result				
			IPM	MEM	Water	ETP	ETP+DPA	ETP+AVI	ETP+VAB
Wild type	*P. aeruginosa* (3)	None	1–2	0.25–0.75	+	–	–	–	–
	P. putida (1)	None	0.5	3	+	–	–	–	–
AmpC overproduction	*P. aeruginosa* (4)	AmpC	0.12–>32	0.19–>32	+	–	–	–	–
Efflux overexpression	*P. aeruginosa* (3)	MexX/Y++	1–1.5	0.5–1	+	–	–	–	–
	P. aeruginosa (3)	MexA/B ++	0.125–>32	1.5–3	+	–	–	–	–
	P. aeruginosa (1)	MexC/D ++	>32	16–>32	+	–	–	–	–
	P. aeruginosa (1)	MexA/B-OprM+	1.5	1.5	+	–	–	–	–
	P. aeruginosa (1)	MexE/F+	>32	3	+	–	–	–	–
Porin deficiency	*P. aeruginosa* (8)	OprD deficient	1.5–>32	2–>32	+	–	–	–	–
Porin deficiency and efflux overexpression	*P. aeruginosa* (1)	OprD deficient, MexA/B-OprM + MexC/D Opr +	4	8	+	–	–	–	–
	P. aeruginosa (1)	OprD deficient, MexA/B-OprM+	4	4	+	–	–	–	–
	P. aeruginosa (1)	OprD deficient, MexA/B-OprM + + ; MexX/Y-OprM+	16	32	+	–	–	–	–
Porin deficiency and AmpC overproduction	*P. aeruginosa* (14)	OprD deficient + AmpC	4–32	4–32	+	–	–	–	–
Porin deficiency, AmpC overproduction, and efflux overexpression	*P. aeruginosa* (3)	OprD deficient AmpC + MexA/B-OprM +	16–32	4–32	+	–	–	–	–
	P. aeruginosa (2)	OprD deficient+AmpC + MexX/Y-OprM +	16	8–32	+	–	–	–	–
	P. aeruginosa (1)	OprD deficient + AmpC + MexC/D-OprJ +	8	4	+	–	–	–	–
	P. aeruginosa (6)	OprD deficient AmpC + MexX/Y-OprM, + MexC/D-OprJ+	16–32	8	+	–	–	–	–
	P. aeruginosa (1)	OprD deficient AmpC + MexA/B-OprM+, MexX/Y-OprM+	64	64	+	–	–	–	–
	P. aeruginosa (2)	OprD deficient + AmpC + MexA/B-OprM, MexC/D-OprJ +	16	8	+	–	–	–	–
Expanded-spectrum-ß-lactamase	*P. aeruginosa* (1)	TEM-4	3	0.75	+	–	–	–	–
	P. aeruginosa (3)	SHV−2a	1.5–3	1–3	+	–	–	–	–
	P. aeruginosa (2)	SHV-5	2	1.5–2	+	–	–	–	–
	P. aeruginosa (7)	PER-1	0.75–>32	0.19–32	+	–	–	–	–
	P. aeruginosa (12)	VEB-1	1–>32	0.125–>32	+	–	–	–	–
	P. aeruginosa (2)	BEL-1	1.5	0.25–0.75	+	–	–	–	–
	P. aeruginosa (1)	BEL-2	1.5	1	+	–	–	–	–
	P. aeruginosa (2)	GES-1	1	0.5–0.75	+	–	–	–	–
	P. aeruginosa (1)	GES-9	2	1	+	–	–	–	–
	P. aeruginosa (1)	OXA-4	0.016	0.19	+	–	–	–	–
	P. aeruginosa (1)	OXA-10	2	1.5	+	–	–	–	–
	P. aeruginosa (3)	OXA-13	2	1.5–2	+	–	–	–	–
	P. aeruginosa (1)	OXA-14	2	2	+	–	–	–	–
	P. aeruginosa (2)	OXA-18	2–>32	2–>32	+	–	–	–	–
	P. aeruginosa (1)	OXA-28	2	0.75	+	–	–	–	–
	P. aeruginosa (3)	OXA-32	>32	4–12	+	–	–	–	–
	P. aeruginosa (1)	OXA-35	2	1	+	–	–	–	–
	P. aeruginosa (1)	OXA-50	2	0.75	+	–	–	–	–

MIC = Minimum Inhibition Concentration; IPM = Imipenem; MEM = meropenem; DPA = dipiclonic acid; AVI = avibactam; VAB = Vaborbactam; **+**, change of solution color from yellow to red; –, no change of color remaining yellow.

efficiently detected acquired carbapenemase production among *Pseudomonas* spp. other than *P. aeruginosa*.

The NitroSpeed-Carba NP test, which cost was evaluated to be ca. 5 US$ per strain to be tested, may easily replace all those tests used for in vitro detection of carbapenemases (such as modified Hodge test, carbapenem inactivation method [CIM]), or those aiming to identify the carbapenemases at the class level, including inhibitor-based methods (EDTA, boronic acid, metallo-β-lactamase Etest) that require 12 to 48 hours to be performed (Pournaras et al., 2010; Walsh et al., 2002). Actually, the NitroSpeed-Carba NP test turnaround time being 30 minutes is much faster than that of the phenotypic methods such as the modified CIM (Sfeir et al., 2019) or its derivative such as the rapid carbapenemase detection method that require 5 to 6 hours to 24 hours to be performed (Jing et al., 2019). The Food and Drug Administration recommends also methods such as matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for carbapenemase detection (Burckhardt and Zimmermann, 2011) that is somehow difficult to implement in routine clinical laboratories.

The rapid immunodetection of carbapenemase producers is another technique that is widely used for detection of carbapenemase activity (Potron et al., 2019; Kieffer et al., 2019). It detects KPC, several IMP variants (not all), VIM, NDM, and OXA-48-like enzymes. However, the use of this technique for detecting carbapenemase activity in *Pseudomonas* spp. may lead to many false-negative results, considering the large variety of enzymes being acquired by those species (GES, GIM, DIM, SPM, and AIM), with the GES-5 enzyme being commonly identified. Molecular detection of carbapenemase encoding genes is an interesting alternative but remains costly and time consuming.

Finally, the NitroSpeed-Carba NP test will be helpful for detecting patients infected or colonized with carbapenemase producers, which is of critical concern in term of optimization of the antibiotic stewardship and prevention of outbreaks. It is very important to highlight that no modification of the test was required for testing *Pseudomonas* spp. isolates, by comparison to the original NitroSpeed-Carba NP test developed for Enterobacterales, which makes it even more interesting for its implementation in clinical laboratories.

Funding

This work was funded by the Swiss National Science Foundation (projects number FNS-407240_177381 and FNS-407240_177382).

Authors Statement

LP and PN designed the study. MS performed the experiments. MS drafted the manuscript. LP and PN wrote the final version of the manuscript.

Conflicts of interest

None.

Ethical approval

Not required

Acknowledgments

We are grateful to Timothy Walsh and Neil Woodford for sharing the AIM- and OXA-181-producing strains, respectively.

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