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MECHANISMS OF RESISTANCE

Evaluation of the Immunochromatographic NG-Test Carba 5 for Rapid Identification of Carbapenemase in Nonfermenters

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ABSTRACT The immunochromatographic assay NG-Test Carba 5 (NG-Biotech) was evaluated with a collection of 107 carbapenemase-producing nonfermenters (CP-NF) (55 *Pseudomonas* spp., 51 *Acinetobacter* spp., and 1 *Achromobacter xylosoxidans* isolate) and 61 carbapenemase-negative isolates. All KPC, VIM, and NDM carbapenemase producers tested were accurately detected. Of the 16 IMP variants tested, 6 (37.5%) variants were not detected. Considering the epidemiology of CP-NFs in France, the NG-Test Carba 5 would detect 89.4% of CP *Pseudomonas* spp. but only 12.9% of CP *Acinetobacter* spp.

KEYWORDS *Acinetobacter*, IMP, KPC, NDM, *Pseudomonas aeruginosa*, VIM, carbapenemases, rapid diagnostic

The production of carbapenemases among *Pseudomonas* and *Acinetobacter* species has become a noteworthy mechanism for multidrug resistance. Whereas carbapenem resistance is often associated with nontransferable mechanisms, such as porin (OprD) deficiency in *Pseudomonas aeruginosa*, most carbapenem-resistant *Acinetobacter* isolates produce a carbapenemase (1, 2). Carbapenemases belong to three classes (A, B, and D) according to the Ambler classification (3). While class B metallo-β-lactamases of the VIM type and IMP type are predominant in *P. aeruginosa* isolates, carbapenem-hydrolyzing class D β-lactamases (CHDL) of the OXA-23-, OXA-24/40, OXA-58, and OXA-143 groups are still the most prevalent carbapenem resistance determinants in *Acinetobacter baumannii*, but NDM producers are increasingly being reported worldwide (2, 4–6). In contrast, KPC ambler class A enzymes and OXA-48-like β-lactamases remain relatively uncommon in nonfermentative Gram-negative species (7–9). In this context, the rapid detection of carbapenemase-producing bacteria has become crucial to prevent their dissemination.

Recently, the NG-Test Carba 5 immunochromatographic assay (ICA) (NG Biotech, Guipry, France) was developed to detect the five most widespread carbapenemase families in carbapenemase-producing *Enterobacterales* (CPEs) (i.e., KPC-, NDM-, VIM-, IMP-, and OXA-48-like enzymes). It was demonstrated to accurately identify the claimed enzymes (10). Compared with other ICAs developed to detect CPEs (RESIT-4 O.K.V.N.; Coris BioConcept, Belgium), the NG-Test Carba 5 targets in addition the very heterogeneous family of IMP-type enzymes, which are more prevalent in nonfermenters than in *Enterobacterales* (11). Here, we have evaluated the performance of the NG-Test Carba 5 for the detection of carbapenemase-producing *Pseudomonas* spp. and *Acinetobacter* spp. encountered in France.

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TABLE 1 Results of the NG-Test Carba 5 on the collection of *Pseudomonas* spp. and *Acinetobacter* spp.[a]

Category	Species	Carbapenemase	No. of isolates	NG-Test Carba 5 result for strains with:					% sensitivity (95% CI)	% specificity (95% CI)
				NDM	IMP	VIM	OXA	KPC		
Carbapenemase producers										
KPC type	*P. aeruginosa*	KPC-2	3	N	N	N	N	**P**	100 (31.0–100)	100 (97.2–100)
GES type	*P. aeruginosa*	GES-2	1	N	N	N	N	N		
	P. aeruginosa	GES-5	1	N	N	N	N	N		
	P. aeruginosa	GES-6	1	N	N	N	N	N		
	A. baumannii	GES-14	1	N	N	N	N	N		
VIM type	*A. xylosoxidans*	VIM-1	1	N	N	**P**	N	N	100 (81.5–100)	100 (96.8–100)
	P. aeruginosa	VIM-1	1	N	N	**P**	N	N		
	P. fluorescens	VIM-2	1	N	N	**P**	N	N		
	P. stutzeri	VIM-2	1	N	N	**P**	N	N		
	P. putida	VIM-2	1	N	N	**P**	N	N		
	P. aeruginosa	VIM-2	9	N	N	**P**	N	N		
	P. aeruginosa	VIM-4	1	N	N	**P**	N	N		
	Acinetobacter genomosp. 16	VIM-4	1	N	N	**P**	N	N		
	A. pittii	VIM-4	1	N	N	**P**	N	N		
	P. aeruginosa	VIM-6	1	N	N	**P**	N	N		
	P. aeruginosa	VIM-11	1	N	N	**P**	N	N		
	P. aeruginosa	VIM-28	1	N	N	**P**	N	N		
	P. aeruginosa	VIM-30	1	N	N	**P**	N	N		
	P. putida	VIM-60	1	N	N	**P**	N	N		
IMP type	*P. stutzeri*	IMP-1	1	N	**P**	N	N	N	62.5 (40.8–80.4)	100 (96.8–100)
	P. putida	IMP-1	1	N	**P**	N	N	N		
	P. aeruginosa	IMP-1	1	N	**P**	N	N	N		
	A. baumannii	IMP-1	1	N	**P**	N	N	N		
	A. pittii	IMP-1	1	N	**P**	N	N	N		
	P. aeruginosa	IMP-2	1	N	**P**	N	N	N		
	A. baumannii	IMP-4	1	N	**P**	N	N	N		
	P. aeruginosa	IMP-5	1	N	**P**	N	N	N		
	A. pittii	IMP-5	1	N	**P**	N	N	N		
	P. aeruginosa	IMP-10	1	N	**P**	N	N	N		
	P. aeruginosa	IMP-13	3	N	N	N	N	N		
	P. aeruginosa	IMP-15	1	N	N	N	N	N		
	P. aeruginosa	IMP-18	1	N	N	N	N	N		
	P. aeruginosa	IMP-19	1	N	**P**	N	N	N		
	P. aeruginosa	IMP-26	1	N	**P**	N	N	N		
	P. aeruginosa	IMP-29	1	N	**P**	N	N	N		
	P. aeruginosa	IMP-39	1	N	**P**	N	N	N		
	P. putida	IMP-63	1	N	N	N	N	N		
	P. aeruginosa	IMP-63	1	N	N	N	N	N		
	P. aeruginosa	IMP-71	1	N	N	N	N	N		
	P. aeruginosa	IMP-79	1	N	**P**	N	N	N		
NDM type	*P. aeruginosa*	NDM-1	2	**P**	N	N	N	N	At 15 min, 92.0 (72.4–98.6); at 30 min, 100 (83.4–100)	At 15 min, 100 (96.8–100); at 30 min, 100 (96.8–100)
	A. baumannii	NDM-1	13	**P**	N	N	N	N		
	A. baumannii	NDM-1	2	N[b]	N	N	N	N		
	A. baumannii	NDM-2	1	**P**	N	N	N	N		
	A. baumannii	NDM-9	1	**P**	N	N	N	N		
OXA type	*P. aeruginosa*	OXA-198	1	N	N	N	N	N		
	A. baumannii	OXA-23	5	N	N	N	N	N		
	A. baumannii	OXA-25	1	N	N	N	N	N		
	A. baumannii	OXA-26	1	N	N	N	N	N		
	A. baumannii	OXA-72	5	N	N	N	N	N		
	A. baumannii	OXA-58	4	N	N	N	N	N		
	A. baumannii	OXA-92	1	N	N	N	N	N		
	A. baumannii	OXA-97	1	N	N	N	N	N		
	A. nosocomialis	OXA-420	1	N	N	N	N	N		

(Continued on next page)

TABLE 1 (Continued)

Category	Species	Carbapenemase	No. of isolates	NG-Test Carba 5 result for strains with:					% sensitivity (95% CI)	% specificity (95% CI)
				NDM	IMP	VIM	OXA	KPC		
Other-carbapenemaseproducers	*P. aeruginosa*	GIM-1	2	N	N	N	N	N		
	P. aeruginosa	AIM-1	2	N	N	N	N	N		
	P. aeruginosa	SPM-1	1	N	N	N	N	N		
	P. aeruginosa	DIM-1	1	N	N	N	N	N		
	A. baumannii	SIM-1	1	N	N	N	N	N		
	P. otitidis	POM-1	1	N	N	N	N	N		
	P. alcaligenes	PAM-1	1	N	N	N	N	N		
Multiple-carbapenemase producers	*A. baumannii*	OXA-23 + NDM-1	6	**P**	N	N	N	N		
	A. junii	IMP-37 + OXA-58	1	N	N	N	N	N		
Non-carbapenemase producers	*P. aeruginosa*		50	N	N	N	N	N		
	A. baumannii		11	N	N	N	N	N		

[a]**P**, positive; N, negative; 95% CI, 95% confidence interval, calculated using online VassarStats software (http://vassarstats.net/).
[b]The NDM band was visible at 30 min but not at 15 min.

A collection of 168 nonfermenters with PCR-characterized β-lactamase content was used to evaluate the ICA NG-Test Carba 5. Those strains were identified to the species level using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MALDI Biotyper CA system; Bruker Daltonics, Billerica, MA, USA) and had previously been characterized at the molecular level for their carbapenemase content (12, 13). This collection included 105 *Pseudomonas* spp. (*P. aeruginosa*, $n = 96$; *P. putida*, $n = 4$; *P. stutzeri*, $n = 2$; *P. fluorescens*, $n = 1$; *P. alcaligenes* $n = 1$; *P. otitidis* $n = 1$), 62 *Acinetobacter* spp. (*A. baumannii*, $n = 56$; *A. pittii*, $n = 3$; *A. junii*, $n = 1$; *Acinetobacter* genomosp. 16, $n = 1$; *A. nosocomialis*, $n = 1$), and 1 *Achromobacter xylosoxidans* strain. Among these 168 isolates, 107 were carbapenemase producers (55 *Pseudomonas* spp., 51 *Acinetobacter* spp., and 1 *A. xylosoxidans* strain) and 61 were not carbapenemase producers (50 *Pseudomonas* spp. and 11 *Acinetobacter* spp.). The produced carbapenemases included 3 KPC enzymes and 71 metallo-β-lactamases targeted by the NG-Test Carba 5 (22 VIM, 24 IMP, and 25 NDM enzymes). Thirty-three isolates produced a carbapenemase not targeted by the NG-Test Carba 5 (4 of the GES type, 5 OXA-23 type, 7 OXA-24/40 type, 7 OXA-58 type, 2 GIM-1, 2 AIM-1, 1 SPM-1, 1 DIM-1, 1 PAM-1, 1 POM-1, 1 OXA-198, and 1 SIM-1). According to the EUCAST guidelines (14), the majority of the carbapenemase-producing strains were not susceptible to imipenem (101/107, 94.4%) or to meropenem (102/107, 95.3%) (see Table S1 in the supplemental material).

The NG-Test Carba 5 identified all KPC ($n = 3$) and VIM ($n = 22$) producers, with no false-positive results. Among the NDM-positive isolates, 92% ($n = 23/25$) were correctly identified. The two isolates that yielded negative results after 15 min of incubation (manufacturer's recommendations) became slightly positive after 30 min. The test allowed the detection of 62.5% of the IMP producers ($n = 15/24$). As has already been reported, the detection of IMP-type carbapenemases remains challenging considering the high sequence diversity within the IMP family (15). Here, 10 out of the 16 different IMP variants were correctly detected. The false-negative results correspond to the IMP-13 clade (IMP-13 and IMP-37), IMP-15, the IMP-18 clade (IMP-18 and IMP-71), and IMP-63 (Table 1, Fig. S1). All 61 non-carbapenemase-producing isolates and all the 33 isolates producing a carbapenemase different from those targeted by the NG-Test Carba 5 gave negative test results, demonstrating no cross-reactivity between OXA CHDLs encountered in *Acinetobacter* (OXA-23, -24/-40, and -58) and OXA-48-like enzymes identified in *Enterobacterales*.

In countries where the epidemiology of carbapenemase-positive *P. aeruginosa* strains is largely dominated by VIM producers and where the incidence of IMP producers is quite low (8% in 2017 in France [P. Plésiat, unpublished results]), it seems reasonable to recommend the use of the NG-Test Carba 5. Considering the French

epidemiology of CP *P. aeruginosa* strains in 2017, the NG-Test Carba 5 might have correctly detected 89.4% of them. However, phenotypic methods (e.g., carbapenem hydrolysis tests, such as the RAPIDEC Carba NP) are still currently required for strains highly suspected of producing a metallo-β-lactamase (carbapenem resistance and high-level resistance to ceftolozane-tazobactam) that produce a negative result by the current version of the NG-Test Carba 5. Of note, the future version of the NG-Test Carba 5 that will be commercialized in 2019 will include all these undetected IMP variants (the IMP-13 clade [IMP-13 and IMP-37], IMP-14, IMP-15, the IMP-18 clade [IMP-18 and IMP-71], and IMP-63 [L. Dortet, unpublished results]), which might be helpful for the accurate detection of carbapenemase producers in countries where IMP producers are more prevalent (e.g., Japan, South Korea, and Taiwan).

In summary, the NG-Test Carba 5 is a useful tool for the accurate identification of CP *Pseudomonas* spp. Since the most prevalent carbapenemases identified in *Acinetobacter* spp. are not targeted by the assay, implementation of this test to identify CP *Acinetobacter* spp. is not recommended or should at least be combined with the OXA-23 K-Set ICA from Coris BioConcept (16).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00968-19.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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