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Rapid Detection of Carbapenemase-Producing *Pseudomonas* spp.

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A novel biochemical technique, the Carba NP test, has been evaluated to detect carbapenemase production in *Pseudomonas* spp. This test was specific (100%), sensitive (94.4%), and rapid (<2 h). This cost-effective test, which could be implemented in any microbiology laboratory, offers a reliable technique for identification of carbapenemase-producing *Pseudomonas* spp.

Pseudomonas aeruginosa is intrinsically resistant to a number of β-lactams due to the low permeability of its outer membrane, the constitutive expression of various efflux pumps, and the production of β-lactamases (5). Acquired resistance to broad-spectrum β-lactams is increasingly observed in *P. aeruginosa*. Currently, PER-, VEB-, and GES-type enzymes are the most frequently observed extended-spectrum β-lactamases (ESBLs) identified in *Pseudomonas* spp. (5, 7). Therefore, carbapenems are considered crucial for treating many *P. aeruginosa*-associated infections.

In *Pseudomonas* spp., carbapenem resistance may be related either to a decreased bacterial outer membrane permeability (e.g., loss or modification of the OprD2 porin or overexpression of efflux pumps), often associated with overexpression of β-lactamases possessing no significant carbapenemase activity (AmpCs), or to expression of true carbapenemases (5, 14). In *Pseudomonas* spp., those carbapenemases are mostly metallo-β-lactamases (MBLs) of the VIM, IMP, SPM, GIM, AIM, DIM, and NDM types and, to a lesser extent, Ambler class A carbapenemases of the KPC and GES types (GES-2, -4, -5, -6, and -11) (2, 3, 12).

Screening of carbapenemase producers among carbapenem-resistant *P. aeruginosa* isolates is important since many carbapenemase genes are plasmid carried and easily transferable. Phenotypic techniques for *in vitro* identification of carbapenemase

Received 15 June 2012 Returned for modification 1 August 2012
Accepted 5 September 2012
Published ahead of print 12 September 2012
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doi:10.1128/JCM.01597-12

TABLE 1 Detection of carbapenemase activity in carbapenemase producers by using the Carba NP test

Ambler class	Carbapenemase type	Organism	β-Lactamase	MIC (mg/liter)		Carba NP test result
				IMP	MER	
A	KPC	*P. aeruginosa* COL	KPC-2	>32	>32	+
		P. aeruginosa P13	KPC-2	>32	>32	+
		P. aeruginosa PA-2	KPC-2	>32	>32	+
		P. aeruginosa PA-3	KPC-2	>32	>32	+
	GES	*P. aeruginosa* GW-1	GES-2	3	1	−
		P. aeruginosa P35	GES-5	>32	>32	−
B	VIM	*P. aeruginosa* P0510	VIM-1	>32	>32	+
		Pseudomonas fluorescens COU	VIM-2	>32	>32	+
		P. aeruginosa REZ	VIM-2	>32	>32	+
		P. putida 9335	VIM-2	>32	>32	+
		P. stutzeri P511503100	VIM-2	>32	>32	+
		P. aeruginosa BY25753	VIM-2	>32	>32	+
		P. aeruginosa V919005	VIM-2	>32	>32	+
		P. aeruginosa AK5493	VIM-2	>32	>32	+
		P. aeruginosa KA-209	VIM-2	>32	>32	+
		P. putida NTU 91/99	VIM-2	>32	>32	+
		P. aeruginosa CAS	VIM-4	>32	>32	+
		P. aeruginosa JAC	VIM-4	>32	>32	+
	IMP	*P. aeruginosa* 12870	IMP-1	12	>32	+
		P. stutzeri PB207	IMP-1	2	4	+
		P. putida NTU 92/99	IMP-1	1	0.19	+
		P. aeruginosa	IMP-1	>32	>32	+
		P. aeruginosa 0607097	IMP-2	>32	>32	+
		P. aeruginosa ITA	IMP-13	>32	>32	+
	NDM	*P. aeruginosa* 453	NDM-1	>32	>32	+
		P. aeruginosa 353	NDM-1	>32	>32	+
	GIM	*P. aeruginosa* 73-12198	GIM-1	3	0.19	+
		P. aeruginosa 73-15574	GIM-1	>32	>32	+
		P. aeruginosa 73-15553A	GIM-1	>32	>32	+
		P. aeruginosa 73-5674	GIM-1	>32	>32	+
	AIM	*P. aeruginosa* WCH2677	AIM-1	>32	>32	+
		P. aeruginosa WCH2813	AIM-1	>32	>32	+
		P. aeruginosa WCH2837	AIM-1	>32	>32	+
	SPM	*P. aeruginosa* 16	SPM-1	>32	>32	+
	DIM	*P. stutzeri* 13	DIM-1	>32	>32	+
	BIC	*P. fluorescens*	BIC-1	>32	4	+

production, such as the modified Hodge test, are not highly sensitive and specific (10). Detection of MBL and KPC producers may be based on the inhibitory properties of several molecules (EDTA and boronic acid, respectively) and requires a significant degree of expertise (9). Indeed, inhibition of carbapenemase activity is more difficult to show in *P. aeruginosa* than in *Enterobacteriaceae* due to its low outer membrane permeability (11). Molecular detection of carbapenemase genes is an interesting alternative but remains costly and also requires a high degree of expertise that is not available for nonspecialized laboratories (16). Both the phenotypic and molecular techniques are time-consuming and therefore do not correspond to the real clinical need. However, the detection of carbapenemase producers must actually be followed by a rapid adaptation of the antibiotic therapy and by the isolation of colonized patients in order to prevent the development of nosocomial outbreaks (6).

The aim of this study was to determine the value of the newly developed Carba NP test (8) to discriminate between carbapenemase- and non-carbapenemase-producing isolates among *Pseudomonas* spp.

The Carba NP test is based on biochemical detection of the hydrolysis of the β-lactam ring of a carbapenem, imipenem, followed by color change of a pH indicator (Fig. 1). This test was performed on strains grown on Mueller-Hinton agar plates (Becton, Dickinson, Le Pont de Chaix, France) at 37°C for 18 to 22 h (8). Briefly, one calibrated loop (10 μl) of the tested strain directly recovered from the Mueller-Hinton agar plate was resuspended in 100 μl of a 20 mM Tris-HCl lysis buffer (bacterial protein extraction reagent II [B-PER II]; Pierce, Thermo Scientific, Villebon-sur-Yvette, France), vortexed for 1 min, and further incubated at room temperature for 30 min. This bacterial suspension was centrifuged at 10,000 × *g* at room temperature for 5 min. Thirty microliters of the supernatant, corresponding to the enzymatic bacterial suspension, was mixed in a well of a 96-well tray with 100 μl of a 1-ml solution made of 3 mg of imipenem monohydrate (Sigma, Saint-Quentin-Fallavier, France) (pH 7.8) phenol red solution and 0.1 mM $ZnSO_4$ (Merck Millipore, Guyancourt, France). The phenol red solution used was prepared by taking 2 ml of a phenol red (Merck Millipore) solution (0.5%, wt/vol) to which 16.6 ml of distilled water was added. The pH value was then adjusted to 7.8 by adding drops of 1 N NaOH. A mixture of the phenol red solution and the enzymatic suspension being tested was incubated at 37°C for a maximum of 2 h. Test results were interpreted by technicians who were blinded to the identity of the samples.

Thirty-six carbapenemase-producing isolates belonging to several *Pseudomonas* species, isolated from various clinical samples and of global origin, have been included in this study (Table 1). The strains had been previously characterized for their β-lactamase content at the molecular level. This collection also contained 72 strains representative of the main β-lactam resistance phenotypes and β-lactamase diversity identified in *Pseudomonas* spp. (including ESBLs of PER, VEB, BEL, SHV, TEM, and OXA types) (Table 2). In addition, most of those strains were resistant to carbapenems.

The Carba NP test differentiated the carbapenemase producers, with the exception of several GES-type producers (Table 1 and Fig. 1), from those isolates that were carbapenem resistant due to non-carbapenemase-mediated mechanisms (the most frequent ones) such as combined mechanisms of resistance (outer membrane permeability defect associated or not with overproduction of cephalosporinase and/or ESBLs) (Table 2). The specificity and sensitivity of the test were found to be 100% and 94.4%, respectively. Interestingly, carbapenemase activity was detected in the two carbapenemase producers (IMP-1-producing *Pseudomonas stutzeri* PB207 and *Pseudomonas putida* NTU 92/99) that were basically susceptible to imipenem (MIC, ≤2 μg/ml) according to the CLSI guidelines (1) (Table 1).

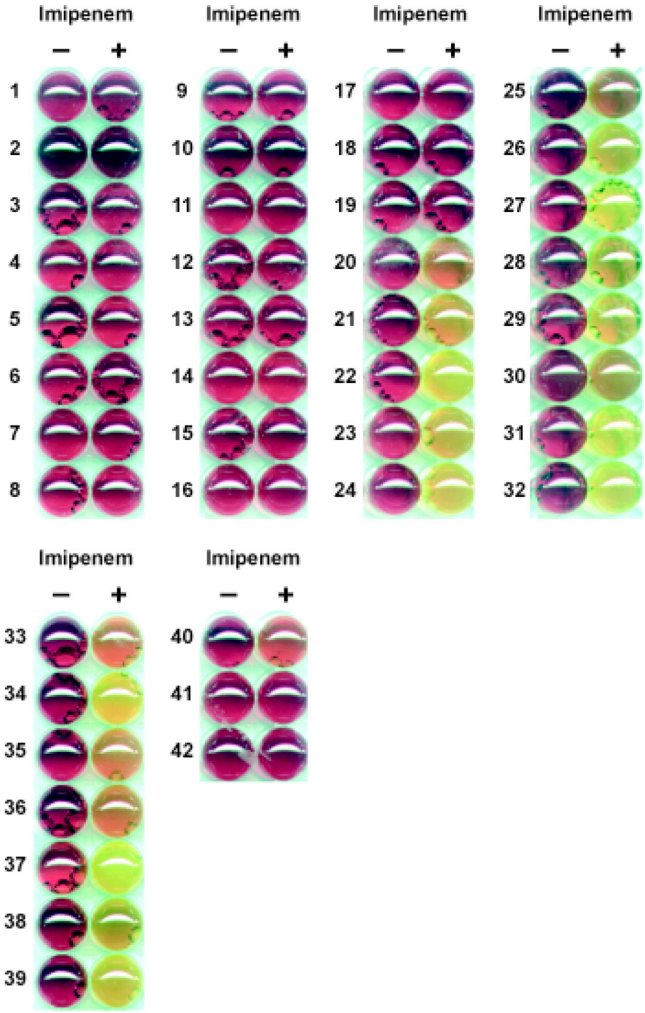

FIG 1 Examples of results for non-carbapenemase-producing and carbapenemase-producing *Pseudomonas* spp. from the Carba NP test. Non-carbapenemase producers are as follows: *P. aeruginosa* 76110, wild type, *P. aeruginosa* PU 21, wild type; *P. aeruginosa* ATCC 27853, wild type; *P. aeruginosa* PAO1, wild type; *P. aeruginosa* HT29, OprM porin deficient; *P. aeruginosa* PAO1, OprD porin deficient; *P. aeruginosa* PAO1, overexpressing MexC/D-OprM efflux pump; *P. aeruginosa*, overexpressing MexA/B-OprM efflux pump; *P. aeruginosa* PAO1, overexpressing MexX/Y-OprM efflux pump; *P. aeruginosa* 3-12, overexpressing the chromosomal AmpC; *P. aeruginosa*, GES-1; *P. aeruginosa*, GES-9; *P. aeruginosa* ED, OXA-28; *P. aeruginosa*, OXA-32; *P. aeruginosa*, SHV-2a; *P. aeruginosa*, SHV-5; *P. aeruginosa*, PER-1; *P. aeruginosa*, VEB-1; and *P. aeruginosa*, BEL-1 (rows 1 to 19, respectively). Carbapenemase producers are as follows; *P. aeruginosa* 16, SPM-1; *P. stutzeri*, DIM-1; *P. aeruginosa* P13, KPC-2; *P. aeruginosa* PA-2, KPC-2; *P. aeruginosa*, VIM-1; *Pseudomonas fluorescens* COU, VIM-2; *P. aeruginosa* REZ, VIM-2; *P. putida*, VIM-2; *P. stutzeri*, VIM-2; *P. aeruginosa* CAS, VIM-4; *P. aeruginosa* JAC, VIM-4; *P. aeruginosa* 1287, IMP-1; *P. stutzeri* PB207, IMP-1; *P. putida* NTU 92/99, IMP-1; *P. aeruginosa* 0607097, IMP-2; *P. aeruginosa*, IMP-13; *P. aeruginosa* 453, NDM-1; *P. aeruginosa* 353, NDM-1; *P. aeruginosa* 73-56, GIM-1; *P. aeruginosa* WCH2677, AIM-1; *P. fluorescens*, BIC-1; *P. aeruginosa* GW-1, GES-2; and *P. aeruginosa*, GES-5 (rows 20 to 42, respectively). Note that strains in rows 41 and 42 gave false-negative results.

TABLE 2 Results of Carba NP test on non-carbapenemase-producing *Pseudomonas* spp.

Resistance mechanism(s)[a]	Organism	Resistance determinant(s)[b]	MIC (mg/liter)		Carba NP test result
			IMP	MER	
Wild type	*P. aeruginosa* 76110	None	0.75	0.19	−
	P. aeruginosa PU21	None	1.5	0.75	−
	P. aeruginosa ATCC 27853	None	2	0.25	−
	P. aeruginosa PAO1	None	1	0.5	−
	P. putida CIP 55-5	None	0.5	3	−
AmpC overproduction	*P. aeruginosa* 3-12	**AmpC**	3	0.25	−
	P. aeruginosa VED	**AmpC**	0.12	0.19	−
Efflux	*P. aeruginosa* PAO1	MexC/D-OprJ	>32	4	−
	P. aeruginosa PT629	MexA/B-OprM	1.5	1.5	−
	P. aeruginosa PAO1	MexX/Y-OprM	1.5	0.75	−
Porin deficiency	*P. aeruginosa* PAO1	OprM deficient	0.75	0.5	−
	P. aeruginosa H729	OprD deficient	>32	6	−
	P. aeruginosa Paeβ-02	OprD deficient	4	4	−
	P. aeruginosa Paeβ-05	OprD deficient	16	8	−
	P. aeruginosa Paeβ-30	OprD deficient	8	8	−
	P. aeruginosa Paeβ-31	OprD deficient	16	8	−
Porin deficiency and efflux	*P. aeruginosa* Paeβ-19	OprD deficient + MexA/B-OprM	4	4	−
	P. aeruginosa Paeβ-29	OprD deficient + MexA/B-OprM + MexX/Y-OprM	16	32	−
	P. aeruginosa Paeβ-01	OprD deficient + MexX/Y-OprM + MexC/D-OprJ	4	8	−
Porin deficiency and AmpC overproduction	*P. aeruginosa* Paeβ-03	OprD deficient + **AmpC**	16	8	−
	P. aeruginosa Paeβ-12	OprD deficient + **AmpC**	16	8	−
	P. aeruginosa Paeβ-13	OprD deficient + **AmpC**	16	8	−
	P. aeruginosa Paeβ-14	OprD deficient + **AmpC**	16	4	−
	P. aeruginosa Paeβ-16	OprD deficient + **AmpC**	32	4	−
	P. aeruginosa Paeβ-23	OprD deficient + **AmpC**	32	16	−
	P. aeruginosa Paeβ-25	OprD deficient + **AmpC**	8	8	−
	P. aeruginosa Paeβ-26	OprD deficient + **AmpC**	4	4	−
	P. aeruginosa Paeβ-32	OprD deficient + **AmpC**	64	16	−
Porin deficiency, AmpC overproduction, and efflux	*P. aeruginosa* Paeβ-04	OprD deficient + **AmpC** + MexA/B-OprM	16	16	−
	P. aeruginosa Paeβ-24	OprD deficient + **AmpC** + MexA/B-OprM	32	32	−
	P. aeruginosa Paeβ-28	OprD deficient + **AmpC** + MexA/B-OprM	16	4	−
	P. aeruginosa Paeβ-15	OprD deficient + **AmpC** + MexX/Y-OprM	16	8	−
	P. aeruginosa Paeβ-21	OprD deficient + **AmpC** + MexX/Y-OprM	16	32	−
	P. aeruginosa Paeβ-22	OprD deficient + **AmpC** + MexC/D-OprJ	8	4	−
	P. aeruginosa Paeβ-06	OprD deficient + **AmpC** + MexX/Y-OprM + MexC/D-OprJ	16	8	−
	P. aeruginosa Paeβ-07	OprD deficient + **AmpC** + MexX/Y-OprM + MexC/D-OprJ	16	8	−
	P. aeruginosa Paeβ-08	OprD deficient + **AmpC** + MexX/Y-OprM + MexC/D-OprJ	16	8	−
	P. aeruginosa Paeβ-09	OprD deficient + **AmpC** + MexX/Y-OprM + MexC/D-OprJ	16	8	−
	P. aeruginosa Paeβ-11	OprD deficient + **AmpC** + MexX/Y-OprM + MexC/D-OprJ	16	8	−
	P. aeruginosa Paeβ-17	OprD deficient + **AmpC** + MexX/Y-OprM + MexC/D-OprJ	32	8	−
	P. aeruginosa Paeβ-18	OprD deficient + **AmpC** + MexA/B-OprM + MexX/Y-OprM	64	64	−
	P. aeruginosa Paeβ-27	OprD deficient + **AmpC** + MexA/B-OprM + MexX/Y-OprM	32	64	−
	P. aeruginosa Paeβ-10	OprD deficient + **AmpC** + MexA/B-OprM + MexC/D-OprJ	16	8	−
	P. aeruginosa Paeβ-20	OprD deficient + **AmpC** + MexA/B-OprM + MexC/D-OprJ	16	8	−
ESBL	*P. aeruginosa* F6R7	GES-1	1	0.75	−
	P. aeruginosa DEJ	GES-9	2	1	−
	P. aeruginosa RNL-1	PER-1	6	6	−
	P. aeruginosa A2O6	PER-1	13	3	−
	P. aeruginosa A5O6	PER-1	1.5	0.38	−
	P. aeruginosa A7O6	PER-1	6	1	−
	P. aeruginosa A3O6	PER-1	3	1.5	−
	P. aeruginosa A8O6	PER-1	>32	12	−
	P. aeruginosa A4O6	PER-1	12	3	−
	P. aeruginosa E3O6	PER-1	>32	12	−
	P. aeruginosa E1O6	PER-1	0.25	0.016	−
	P. aeruginosa C2O7	PER-1	>32	8	−
	P. aeruginosa C1O7	PER-1	>32	>32	−
	P. aeruginosa 15	VEB-1	2	1.5	−
	P. aeruginosa 51170	BEL-1	1	0.5	−
	P. aeruginosa 0602-52025	SHV2-a	1.5	3	−
	P. aeruginosa 1782	SHV-5	2	2	−
	P. aeruginosa SHAM	TEM-4	3	0.75	−
	P. aeruginosa PU 21	OXA-2	2	1	−

(Continued on following page)

TABLE 2 (Continued)

Resistance mechanism(s)[a]	Organism	Resistance determinant(s)[b]	MIC (mg/liter)		Carba NP test result
			IMP	MER	
	P. aeruginosa PAO38	OXA-4	0.016	0.19	−
	P. aeruginosa PU 21	OXA-10	2	1.5	−
	P. aeruginosa PU 21	OXA-11	3	1.5	−
	P. aeruginosa NAJ	OXA-13	2	1.5	−
	P. aeruginosa PU 21	OXA-14	2	2	−
	P. aeruginosa MUS	OXA-18 + OXA-20	>32	>32	−
	P. aeruginosa ED	OXA-28	2	0.75	−
	P. aeruginosa PIC	OXA-31	>32	1.5	−
	P. aeruginosa PG13	OXA-32	>32	12	−

[a] Lack of OprD, AmpC overexpression, and efflux system overproduction were previously characterized by quantitative reverse transcription-PCR (12).
[b] Bold AmpC corresponds to overexpression of chromosomal AmpC. Mex-type efflux systems are indicated when overexpressed.

The Carba NP test has multiple benefits for detecting carbapenemase activity in nonfermenters such as *Pseudomonas* spp. It eliminates the need for *in vitro* detection of carbapenemase activity (Hodge test) and for β-lactamase inhibitor-based phenotypic techniques (boronic acid for KPC and EDTA for MBLs), which both require at least 24 to 72 h to be performed. The Carba NP test is the first technique available to identify carbapenemase producers with such high specificity, sensitivity, and rapidity (less than 2 h). However, the absence of detection of GES-type carbapenemases has to be considered, especially in geographical regions with a high prevalence (i.e., Brazil and South Africa) (although other GES-producing strains shall be tested). The GES-type carbapenemases are point mutant analogues of the ESBL GES-1 that possess an additional and rather weak carbapenemase activity (4) that may explain this lack of detection. In addition, the real clinical significance of the carbapenemase activity of GES-type variants as a source of *in vivo* resistance to carbapenems (therapeutic failure) remains to be evaluated (13, 15).

Using this accurate test would be helpful for detecting patients infected or colonized with carbapenemase producers, which is of utmost importance for better antibiotic stewardship and prevention of outbreaks (6). Use of the Carba NP test may be useful in particular for intensive care unit (ICU) and burn patients, among whom multidrug-resistant *P. aeruginosa* isolates are widespread. It offers a cost-effective solution for detecting carbapenemase producers and preventing their spread, considering that they may harbor those carbapenemase genes on plasmids that can spread to other bacterial families (*Enterobacteriaceae* and the family that includes *Acinetobacter* species).

ACKNOWLEDGMENT

This work was funded by a grant from the INSERM (UMR914).

We thank T. R. Walsh for the gift of the GIM- and AIM-positive isolates, P. Plésiat for the gift of some of the efflux and AmpC overproducers, and B. Kocic for the gift of the NDM-positive isolates.

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