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Bloodstream Infections Caused by *Pseudomonas* spp.: How To Detect Carbapenemase Producers Directly from Blood Cultures

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The Carba NP test has been evaluated to detect carbapenemase-producing *Pseudomonas* spp. directly from blood cultures. This rapid and cost-effective test permits an early identification of carbapenemase-producing *Pseudomonas* spp. directly from blood cultures with excellent sensitivity and specificity. Results may be useful in particular for guiding the first-line therapy and epidemiological purposes.

Pseudomonas aeruginosa shows a remarkable capacity to resist antibiotics. This pathogen is intrinsically resistant to narrow-spectrum β-lactams due to the constitutive expression of β-lactamases and efflux pumps and the low permeability of the outer membrane (1). In addition, acquired extended-spectrum β-lactamases (ESBLs) of the PER, VEB, and GES types have been reported in *P. aeruginosa* (2). Consequently, the treatment of *P. aeruginosa*-related infections is becoming increasingly difficult, and therefore carbapenem-containing treatments are frequently needed to treat those infections.

Carbapenem resistance in *Pseudomonas* spp. can result from different mechanisms, such as 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 (1, 3). Carbapenemases have been reported in *Pseudomonas* spp., including the Ambler class A KPC- and GES-type β-lactamases and most commonly the Ambler class B or metallo-β-lactamases (MBLs) of the VIM, IMP, SPM, GIM, AIM, DIM, FIM, and NDM types (1, 3–7).

Spread of carbapenemase-producing *P. aeruginosa* strains is a critical issue since those strains are resistant to almost all β-lactams. In addition, the early detection of carbapenemases is important because carbapenemase genes are usually located on transferable genetic determinants such as plasmids, leading to their rapid dissemination. Carbapenemase producers are commonly first screened based on susceptibility testing results. Then, specific phenotypical tests may be used to identify carbapenemase production *in vitro*, such as the modified Hodge test (8). Other detection methods based on the inhibitory properties of EDTA (against MBL) and boronic acid or clavulanic acid (for KPC) allow the discrimination of the different carbapenemase types. Since those techniques are performed on strains grown on agar plates, they do require at least an additional 24- to 48-h period of time after the blood culture is flagged as positive, and significant expertise is needed (9), particularly with *P. aeruginosa* (10). Molecular methods remain costly and require substantial expertise (11, 12). Overall, both phenotypic and molecular tests are time-consuming and consequently not suited for routine and rapid testing. One of the most recent and reliable techniques for the detection of carbapenemase-producing *P. aeruginosa* is the biochemically based Carba NP test (11). This test, applied on isolated cultures, detects all types of carbapenemase-producing *P. aeruginosa* isolates with the exception of several GES-type producers (13). In addition, this test may be used for detecting carbapenemase-producing *Enterobacteriaceae* from blood (12).

The aim of this study was to determine the ability of the Carba NP test to detect carbapenemase-producing *Pseudomonas* species directly from blood cultures. Since the successful treatment of bacteremia depends on prompt administration of the appropriate antibiotherapy, the routine use of the Carba NP test directly on blood culture may guide the first-line therapy for patients with sepsis (13).

The Carba NP test was performed on spiked blood cultures from which the positivity was assessed using the BacT/Alert blood culture system (bioMérieux, Marcy l'Etoile, France). Blood cultures (10 ml of sterile human blood) were inoculated with 1×10^3 CFU of each strain (500-μl total volume) (Table 1). The inoculum of 1×10^3 CFU was prepared by diluting a 0.5 McFarland standard suspension (10^8 CFU/ml) in sterile water. Then, aerobic bottles (BacT/Alert SA standard aerobic bottles without charcoal) were incubated until a positivity of the blood culture was detected by the BacT/Alert system (detection time ranged from 6 to 15 h). As previously described, the final bacterial count at the time of positivity ranged from 5×10^7 to 5×10^9 CFU/ml (14).

The Carba NP test from blood cultures adapted from the previously published techniques (13, 14) was performed as follows: 2 ml brain heart infusion (BHI) supplemented with 70 μg/ml $ZnSO_4$ (final concentration) without imipenem was inoculated with five drops (75 μl) of the positive blood culture in two Eppendorf tubes (tubes A and B). Inoculated BHI was then incubated under agitation at 37°C for 3 h. Bacteria were recovered by centrifugation at 10,000 × *g* for 5 min. This optimized protocol of the Carba NP test was directly performed on this bacterial pellet (15). Briefly, the bacterial pellet was resuspended in 100 μl of a Tris-

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TABLE 1 Carbapenemase-producing strains included in this study and found positive using the Carba NP test

Ambler class	Carbapenemase type	*Pseudomonas* organism	Carbapenemase	MIC (μg/ml)[a]	
				IMP	MER
A	KPC	*P. aeruginosa* COL	KPC-2	>32	>32
	KPC	*P. aeruginosa* P13	KPC-2	>32	>32
	KPC	*P. aeruginosa* PA-2	KPC-2	>32	>32
	KPC	*P. aeruginosa* PA-3	KPC-2	>32	>32
	GES	*P. aeruginosa* GW-1	GES-2	3	1
	GES	*P. aeruginosa* P-35	GES-5	>32	>32
B	VIM	*P. aeruginosa* P0510	VIM-1	>32	>32
	VIM	*P. fluorescens* COU	VIM-1	>32	>32
	VIM	*P. aeruginosa* JOU	VIM-1	>32	>32
	VIM	*P. aeruginosa* ABA	VIM-1	>32	>32
	VIM	*P. aeruginosa* REZ	VIM-2	>32	>32
	VIM	*P. putida* 9335	VIM-2	>32	>32
	VIM	*P. stutzeri* P511503100	VIM-2	>32	>32
	VIM	*P. aeruginosa* BY25753	VIM-2	>32	>32
	VIM	*P. aeruginosa* V919005	VIM-2	>32	>32
	VIM	*P. aeruginosa* AK5493	VIM-2	>32	>32
	VIM	*P. aeruginosa* KA-209	VIM-2	>32	>32
	VIM	*P. putida* NTU 91/99	VIM-2	>32	>32
	VIM	*P. aeruginosa* COL-1	VIM-2	>32	>32
	VIM	*P. aeruginosa* CAS	VIM-2	>32	>32
	VIM	*P. aeruginosa* JAC	VIM-4	>32	>32
	VIM	*P. aeruginosa* MED	VIM-5	>32	>32
	IMP	*P. aeruginosa* 12870	IMP-1	12	>32
	IMP	*P. stutzeri* PB207	IMP-1	2	4
	IMP	*P. putida* NTU 92/99	IMP-1	1	0.19
	IMP	*P. aeruginosa*	IMP-1	>32	>32
	IMP	*P. aeruginosa* MKA	IMP-1	16	>32
	IMP	*P. aeruginosa* 0607097	IMP-2	>32	>32
	IMP	*P. aeruginosa ITA*	IMP-13	>32	>32
	IMP	*P. aeruginosa* PADI	IMP-13	4	3
	IMP	*P. aeruginosa* TSE	IMP-13	>32	>32
	NDM	*P. aeruginosa* 453	NDM-1	>32	>32
	NDM	*P. aeruginosa* 353	NDM-1	>32	>32
	GIM	*P. aeruginosa* 73-15574	GIM-1	>32	>32
	GIM	*P. aeruginosa* 73-15553A	GIM-1	>32	>32
	GIM	*P. aeruginosa* 73-5674	GIM-1	>32	>32
	AIM	*P. aeruginosa* WCH2677	AIM-1	>32	>32
	AIM	*P. aeruginosa* WCH2813	AIM-1	>32	>32
	AIM	*P. aeruginosa* WCH2837	AIM-1	>32	>32
	SPM	*P. aeruginosa* 16	SPM-1	>32	>32
	DIM	*P. stutzeri*13	DIM-1	>32	>32
	FIM	*P. aeruginosa*	FIM-1	>32	>32

[a] IMP, imipenem; MER, meropenem.

HCl-20 mM lysis buffer (B-PER II, bacterial protein extraction reagent; Pierce, Thermo Scientific, Villebon-sur-Yvette, France) and vortexed for 1 min. The enzymatic bacterial suspension was mixed with 100 μl of a diluted phenol red solution containing 0.1 mM $ZnSO_4$ (Merck Millipore, Guyancourt, France) in the first tube (A) and with a diluted phenol red solution containing 0.1 mM $ZnSO_4$ and supplemented with 6 mg/ml imipenem monohydrate (Sigma, Saint-Quentin-Fallavier, France) in the second tube (B). Mixtures of the phenol red (±imipenem) solutions and the enzymatic suspension being tested were incubated at 37°C for a maximum of 2 h. Results of the Carba NP test were interpreted as follows: (i) both tubes A and B red, non-carbapenemase-producing isolate; (ii) tube A red and tube B yellow/orange, carbapenemase-producing isolate; and (iii) tube A yellow/orange and tube B yellow/orange, noninterpretable result. All experiments were performed in triplicate, and test results were interpreted by technicians who were blinded to the identity of the samples.

A panel of 42 carbapenemase-producing *Pseudomonas* species was included in the study (Table 1), and 72 non-carbapenemase-producing *Pseudomonas* species were used as controls (Table 2). All the strains had previously been characterized for their β-lactamase content and carbapenemase resistance mechanisms at the molecular level in previous works (11).

This optimized protocol of Carba NP test (15), performed directly from spiked blood cultures, perfectly differentiated carbapenemase producers from carbapenem-resistant isolates with non-carbapenemase-mediated mechanisms, such as those combining outer membrane permeability defects associated or not with over-

TABLE 2 Lack of detection of carbapenemase activity using the Carba NP test

Resistance mechanism	*Pseudomonas* organism	Resistance determinant(s)	MIC (μg/ml)	
			IMP	MER
Wild type	*P. aeruginosa* 76110	Reference strain	0.75	0.19
	P. aeruginosa PU21	Reference strain	1.5	0.75
	P. aeruginosa ATCC 27853	Reference strain	2	0.25
	P. aeruginosa PA01	Reference strain	1	0.5
	P. putida CIP 55-5	Reference strain	0.5	3
AmpC overproducer	*P. aeruginosa* 3-12	Overexpression of chromosomal AmpC	3	0.25
	P. aeruginosa Ved	Overexpression of chromosomal AmpC	0.12	0.19
Overproduced efflux	*P. aeruginosa* PA01	Mex C/D-OprJ	>32	4
	P. aeruginosa PT629	Mex A/B-OprM	1.5	1.5
	P. aeruginosa PA01	Mex X/Y-OprM	1.5	0.75
Porin deficiency	*P. aeruginosa* PA01	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 overproduced 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, overproduced AmpC, and overproduced 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*	GES-1	1	0.75
	P. aeruginosa DEJ	GES-9	2	1
	P. aeruginosa RNL-1	PER-1	6	6
	P. aeruginosa	PER-1	13	3
	P. aeruginosa	PER-1	1.5	0.38
	P. aeruginosa	PER-1	6	1
	P. aeruginosa	PER-1	3	1.5

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TABLE 2 (Continued)

Resistance mechanism	*Pseudomonas* organism	Resistance determinant(s)	MIC (μg/ml)	
			IMP	MER
	P. aeruginosa	PER-1	>32	12
	P. aeruginosa	PER-1	12	3
	P. aeruginosa	PER-1	>32	12
	P. aeruginosa	PER-1	0.25	0.016
	P. aeruginosa	PER-1	>32	8
	P. aeruginosa	PER-1	>32	>32
	P. aeruginosa 15	VEB-1	2	1.5
	P. aeruginosa 51170	BEL-1	1	0.5
	P. aeruginosa	SHV2a	1.5	3
	P. aeruginosa 1782	SHV-5	2	2
	P. aeruginosa SHAM	TEM-4	3	0.75
	P. aeruginosa PU21	OXA-2	2	1
	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

production of cephalosporinase and/or ESBLs. The sensitivity and specificity of the test were 100% under those conditions. Of note, GES-type producers were well detected from blood cultures, whereas they were not from isolated colonies (11). This difference might be explained by (i) the increased inoculum recovered from blood cultures during experiments, as previously observed for ESBL-producing *Enterobacteriaceae* (14), and (ii) increased β-lactamase production in liquid medium. Furthermore, the test was positive for two strains (IMP-1-producing *Pseudomonas stutzeri* PB207 and *Pseudomonas putida* NTU 92/99) that were susceptible to carbapenems (MIC ≤ 2 μg/ml) (16).

The Carba NP test combines multiple advantages. It is inexpensive, rapid, reproducible, and highly sensitive and specific. Use of this test would be helpful for choosing a first-line therapy based on an aminoglycoside associated with a fluoroquinolone rather than β-lactam-containing combinations in case of positivity, in particular when treating pneumonia. It can also be useful for detecting carbapenemase producers in an attempt to control outbreaks and to rapidly differentiate between carbapenemase producers (transferable resistance determinant) and noncarbapenemase producers (nontransferable resistance determinant). Actually, by preventing their spread, and therefore statistically reducing their genetic exchanges with other bacterial species, early identification of carbapenemase-producing *P. aeruginosa* strains may prevent further dissemination of carbapenemases to *Enterobacteriaceae*. It corresponds, to the best of our knowledge, to the first easy-to-handle technique allowing rapid identification of carbapenemase producers in *P. aeruginosa* from blood.

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