

**Bloodstream infections caused by *Pseudomonas* spp.; how to detect
carbapenemase producers directly from positive blood cultures ?**

Laurent Dortet,¹ Anne Boulanger,¹ Laurent Poirel,^{1,2} and Patrice Nordmann^{1,2*}

*¹INSERM U914 "Emerging Resistance to Antibiotics", South-Paris Medical School, K.-
Bicêtre, France and ²Medical and Molecular Microbiology Unit, Department of Medicine,
Faculty of Science, University of Fribourg, Switzerland*

*Corresponding author : patrice.nordmann@unifr.ch

Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science,
University of Fribourg, rue Albert-Gockel 3, CH-1700 Fribourg, Switzerland
Phone: +41 26 300 9581

Word count: 1,044

Running title; Detection of carbapenemase-producing *Pseudomonas* from blood

Key words: Carba NP test, Gram negatives, β -lactamase, resistance, rapid diagnostic

Abstract

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, efflux pumps combined with 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 over-expression 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 determinant 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 24-48 hours 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 testings. One of the most recent and reliable technique 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 routinely 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 BactAlert 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 μ l total volume) (Table 1). The 1×10^3 inoculum was prepared by diluting 0.5 McFarland 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 BactAlert system (detection time ranged from 6 to 15 h). As previously described, the final bacterial count at 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 ZnSO₄ (final concentration) without imipenem were inoculated with five drops (75 µl) of the positive blood culture in two Eppendorf tubes (tube A and B). Inoculated BHI were then incubated under agitation at 37°C for 3 h. Bacteria were recovered by centrifugation at 10,000 x 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-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 ZnSO₄ (Merck Millipore, Guyancourt, France) in the first tube (A) and with a diluted phenol red solution containing 0.1 mM ZnSO₄ and supplemented with 6 mg/ml imipenem monohydrate (Sigma, Saint-Quentin-Fallavier, France) in the second tube (B). Mixture 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; not interpretable 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 forty-two carbapenemase-producing *Pseudomonas* species was included in the study (Table 1) and seventy-two non-carbapenemase producers *Pseudomonas* 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 with overproduction of cephalosporinase and/or ESBLs. The sensitivity and specificity of the test were 100% under those conditions. Noteworthy, 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 during experiments blood cultures, 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 ($\text{MIC} \leq 2$ $\mu\text{g/ml}$) (16).

The Carba NP test combines multiple advantages. It is inexpensive, rapid, reproducible, 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 non-carbapenemase producers (non-transferable resistance determinant). Actually, by preventing their spread, and therefore statistically reducing their genetic exchanges with other bacterial species, an early identification of carbapenemase-producing *P. aeruginosa* 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.

Acknowledgments

This work was funded by a grant from the INSERM (UMR914) and the University of Fribourg.

We thank T.R. Walsh for the gift of the GIM- and AIM-positive isolates, P. Plésiat for the gift of several efflux and AmpC overproducers, B. Kocic for the gift of the NDM-positive isolates, and G.M. Rossolini for the gift of the FIM-positive isolate.

References

1. **Mesaros N, Nordmann P, Plesiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, Van Laethem Y, Jacobs F, Lebecque P, Malfroot A, Tulkens PM, Van Bambeke F.** 2007. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin. Microbiol. Infect. **13**:560-578.
2. **Nordmann P, Naas T.** 2010. β -Lactams and *Pseudomonas aeruginosa*, p. 157-174. In Courvalin P, Leclercq R, Rice LB (ed.), Antibigram. ASM Press, Portland.
3. **Rodriguez-Martinez JM, Poirel L, Nordmann P.** 2009. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **53**:4783-4788.
4. **Gupta V.** 2008. Metallo- β -lactamases in *Pseudomonas aeruginosa* and *Acinetobacter* species. Expert Opin. Investig. Drugs **17**:131-143.
5. **Jovcic B, Lepsanovic Z, Suljagic V, Rackov G, Begovic J, Topisirovic L, Kojic M.** 2011. Emergence of NDM-1 metallo- β -lactamase in *Pseudomonas aeruginosa* clinical isolates from Serbia. Antimicrob. Agents Chemother. **55**:3929-3931.

6. **Pollini S, Maradei S, Pecile P, Olivo G, Luzzaro F, Docquier JD, Rossolini GM.** 2013. FIM-1, a new acquired metallo- β -lactamase from a *Pseudomonas aeruginosa* clinical isolate from Italy. *Antimicrob. Agents Chemother.* **57**:410-416.
7. **Strateva T, Yordanov D.** 2009. *Pseudomonas aeruginosa* - a phenomenon of bacterial resistance. *J. Med. Microbiol.* **58**:1133-1148.
8. **Pasteran F, Veliz O, Rapoport M, Guerriero L, Corso A.** 2011. Sensitive and specific modified Hodge test for KPC and metallo- β -lactamase detection in *Pseudomonas aeruginosa* by use of a novel indicator strain, *Klebsiella pneumoniae* ATCC 700603. *J. Clin. Microbiol.* **49**:4301-4303.
9. **Pasteran F, Veliz O, Faccone D, Guerriero L, Rapoport M, Mendez T, Corso A.** 2011. A simple test for the detection of KPC and metallo- β -lactamase carbapenemase-producing *Pseudomonas aeruginosa* isolates with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. *Clin. Microbiol. Infect.* **17**:1438-1441.
10. **Picao RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, Gales AC.** 2008. Metallo- β -lactamase detection: comparative evaluation of double-disk synergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. *J. Clin. Microbiol.* **46**:2028-2037.
11. **Dortet L, Poirel L, Nordmann P.** 2012. Rapid detection of carbapenemase-producing *Pseudomonas* spp. *J. Clin. Microbiol.* **50**:3773-3776.
12. **Dortet L, Brechard L, Poirel L, Nordmann P.** 2013. Rapid detection of carbapenemase-producing Enterobacteriaceae from blood cultures. *Clin. Microbiol. Infect.* In press.

13. **Akova M, Daikos GL, Tzouvelekis L, Carmeli Y.** 2012. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. *Clin. Microbiol. Infect.* **18**:439-448.
14. **Nordmann P, Dortet L, Poirel L.** 2012. Rapid detection of extended-spectrum- β -lactamase-producing *Enterobacteriaceae*. *J. Clin. Microbiol.* **50**:3016-3022.
15. **Dortet L, Bréchart L, Poirel L, Nordmann P.** 2013. Comparison of diverse growing media for further detection of carbapenemase production using the Carba NP test. *J. Med. Microbiol.* In press.
16. **Clinical and Laboratory Standards Institute.** 2013. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-third Informational Supplement, M100-S23, vol. M100-S22. CLSI, Wayne (PA), USA.

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

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

MIC, Minimal inhibitory concentration

IMP, imipenem

MER, meropenem

Table 2. Lack of detection of carbapenemase activity using the Carba NP test

Resistance mechanism	Organism	Resistance determinant(s)	MIC (µg/ml)	
			IMP	MER
Wild type	<i>P. aeruginosa</i> 76110	Reference strain	0.75	0.19
	<i>P. aeruginosa</i> PU21	Reference strain	1.5	0.75
	<i>P. aeruginosa</i> ATCC 27853	Reference strain	2	0.25
	<i>P. aeruginosa</i> PA01	Reference strain	1	0.5
	<i>P. putida</i> CIP 55-5	Reference strain	0.5	3
AmpC overproducer	<i>P. aeruginosa</i> 3-12	overexpression of chromosomal AmpC	3	0.25
	<i>P. aeruginosa</i> Ved	overexpression of chromosomal AmpC	0.12	0.19
Overproduced efflux	<i>P. aeruginosa</i> PA01	Mex C/D-OprJ	>32	4
	<i>P. aeruginosa</i> PT629	Mex A/B-OprM	1.5	1.5
	<i>P. aeruginosa</i> PA01	Mex X/Y-OprM	1.5	0.75
Porin deficiency	<i>P. aeruginosa</i> PA01	OprD deficient	0.75	0.5
	<i>P. aeruginosa</i> H729	OprD deficient	>32	6
	<i>P. aeruginosa</i> Paeβ-02	OprD deficient	4	4
	<i>P. aeruginosa</i> Paeβ-05	OprD deficient	16	8
	<i>P. aeruginosa</i> Paeβ-30	OprD deficient	8	8
	<i>P. aeruginosa</i> Paeβ-31	OprD deficient	16	8
Porin deficiency + overproduced efflux	<i>P. aeruginosa</i> Paeβ-19	OprD deficient + MexA/B-OprM	4	4
	<i>P. aeruginosa</i> Paeβ-29	OprD deficient + MexA/B-OprM + MexX/Y-OprM	16	32
	<i>P. aeruginosa</i> Paeβ-01	OprD deficient + MexX/Y-OprM + MexC/D-OprJ	4	8
Porin deficiency + AmpC over-production	<i>P. aeruginosa</i> Paeβ-03	OprD deficient + AmpC	16	8
	<i>P. aeruginosa</i> Paeβ-12	OprD deficient + AmpC	16	8
	<i>P. aeruginosa</i> Paeβ-13	OprD deficient + AmpC	16	8
	<i>P. aeruginosa</i> Paeβ-14	OprD deficient + AmpC	16	4
	<i>P. aeruginosa</i> Paeβ-16	OprD deficient + AmpC	32	4
	<i>P. aeruginosa</i> Paeβ-23	OprD deficient + AmpC	32	16
	<i>P. aeruginosa</i> Paeβ-25	OprD deficient + AmpC	8	8
	<i>P. aeruginosa</i> Paeβ-26	OprD deficient + AmpC	4	4
Porin deficiency + overproduced AmpC + overproduced efflux	<i>P. aeruginosa</i> Paeβ-32	OprD deficient + AmpC	64	16
	<i>P. aeruginosa</i> Paeβ-04	OprD deficient + AmpC + MexA/B-OprM	16	16
	<i>P. aeruginosa</i> Paeβ-24	OprD deficient + AmpC + MexA/B-OprM	32	32
	<i>P. aeruginosa</i> Paeβ-28	OprD deficient + AmpC + MexA/B-OprM	16	4
	<i>P. aeruginosa</i> Paeβ-15	OprD deficient + AmpC + MexX/Y-OprM	16	8

	<i>P. aeruginosa</i> Paeβ-21	OprD deficient + AmpC + MexX/Y-OprM	16	32
	<i>P. aeruginosa</i> Paeβ-22	OprD deficient + AmpC + MexC/D-OprJ	8	4
	<i>P. aeruginosa</i> Paeβ-06	OprD deficient + AmpC + MexX/Y-OprM + MexC/D-OprJ	16	8
	<i>P. aeruginosa</i> Paeβ-07	OprD deficient + AmpC + MexX/Y-OprM + MexC/D-OprJ	16	8
	<i>P. aeruginosa</i> Paeβ-08	OprD deficient + AmpC + MexX/Y-OprM + MexC/D-OprJ	16	8
	<i>P. aeruginosa</i> Paeβ-09	OprD deficient + AmpC + MexX/Y-OprM + MexC/D-OprJ	16	8
	<i>P. aeruginosa</i> Paeβ-11	OprD deficient + AmpC + MexX/Y-OprM + MexC/D-OprJ	16	8
	<i>P. aeruginosa</i> Paeβ-17	OprD deficient + AmpC + MexX/Y-OprM + MexC/D-OprJ	32	8
	<i>P. aeruginosa</i> Paeβ-18	OprD deficient + AmpC + MexA/B-OprM + MexX/Y-OprM	64	64
	<i>P. aeruginosa</i> Paeβ-27	OprD deficient + AmpC + MexA/B-OprM + MexX/Y-OprM	32	64
	<i>P. aeruginosa</i> Paeβ-10	OprD deficient + AmpC + MexA/B-OprM + MexC/D-OprJ	16	8
	<i>P. aeruginosa</i> Paeβ-20	OprD deficient + AmpC + MexA/B-OprM + MexC/D-OprJ	16	8
ESBL	<i>P. aeruginosa</i>	GES-1	1	0.75
	<i>P. aeruginosa</i> DEJ	GES-9	2	1
	<i>P. aeruginosa</i> RNL-1	PER-1	6	6
	<i>P. aeruginosa</i>	PER-1	13	3
	<i>P. aeruginosa</i>	PER-1	1.5	0.38
	<i>P. aeruginosa</i>	PER-1	6	1
	<i>P. aeruginosa</i>	PER-1	3	1.5
	<i>P. aeruginosa</i>	PER-1	>32	12
	<i>P. aeruginosa</i>	PER-1	12	3
	<i>P. aeruginosa</i>	PER-1	>32	12
	<i>P. aeruginosa</i>	PER-1	0.25	0.016
	<i>P. aeruginosa</i>	PER-1	>32	8
	<i>P. aeruginosa</i>	PER-1	>32	>32
	<i>P. aeruginosa</i> 15	VEB-1	2	1.5
	<i>P. aeruginosa</i> 51170	BEL-1	1	0.5
	<i>P. aeruginosa</i>	SHV2a	1.5	3
	<i>P. aeruginosa</i> 1782	SHV-5	2	2
	<i>P. aeruginosa</i> SHAM	TEM-4	3	0.75
	<i>P. aeruginosa</i> PU21	OXA-2	2	1
	<i>P. aeruginosa</i> PAO38	OXA-4	0.016	0.19
	<i>P. aeruginosa</i> PU 21	OXA-10	2	1.5
	<i>P. aeruginosa</i> PU 21	OXA-11	3	1.5
	<i>P. aeruginosa</i> NAI	OXA-13	2	1.5
	<i>P. aeruginosa</i> PU 21	OXA-14	2	2
	<i>P. aeruginosa</i> MUS	OXA-18 + OXA-20	>32	>32
	<i>P. aeruginosa</i> ED	OXA-28	2	0.75
	<i>P. aeruginosa</i> PIC	OXA-31	>32	1.5

