Rapid identification of carbapenemase-producing *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* using a modified Carba NP test

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Abstract

Biochemical tests have been previously developed to identify carbapenemase-producing *Enterobacteriaceae, Pseudomonas* spp. (Carba NP test) and *Acinetobacter* spp. (CarbAcineto NP test). We evaluated a modified Carba NP test to detect carbapenemase production in *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* species using a single protocol with rapid results and found good reliability and speed.

Keywords: Carba NP test, carbapenemase, carbapenems, Gram negative, multidrug-resistant bacteria
Original Submission: 24 June 2015; **Accepted:** 2 July 2015
Available online 10 July 2015

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Multidrug-resistant Gram-negative bacteria (GNB) are increasingly being reported worldwide. The spread of carbapenemase-producing *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* species have become a global threat. The emergence of resistance to carbapenems makes the treatment for infections caused by these carbapenem-resistant strains very limited [1–3]. Different types of carbapenemases have been reported, such as Ambler class A *Klebsiella pneumoniae* carbapenemase (KPC) and Guiana extended spectrum (GES) β-lactamase, Ambler class B metallo-β-lactamases (MBL) and Ambler class D oxacillinase type [1].

Rapid methods for detecting carbapenemase producers have been described, such as the MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) carbapenemase assay [4]. Previous studies have described a rapid biochemical carbapenemase detection method based on imipenem hydrolysis, the Carba NP test, for *Enterobacteriaceae* [5] and *Pseudomonas* species [6], as well as the CarbAcineto NP test for *Acinetobacter* species [7]. Recently, however, several authors have published evaluations of the Carba NP and the CarbAcineto NP tests; their criticisms focussed essentially on the absence of detection of oxacillinase (OXA) type carbapenemases [8–10].

Here we describe a modified Carba NP (MCNP) test which enables the rapid detection of different carbapenemases (KPC, MBL and OXA types) from *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* species using a single protocol.

One hundred ten previously characterized GNB, including 69 carbapenemase-producing GNB (*Enterobacteriaceae n* = 14, *Pseudomonas aeruginosa n* = 11 and *Acinetobacter baumannii n* = 44), and 41 non-carbapenemase-producing GNB, including *Enterobacteriaceae* (*n* = 24), *P. aeruginosa* (*n* = 5) and *A. baumannii* (*n* = 12), were tested in two laboratories including Unité de recherche sur les maladies infectieuses et tropicales émergentes (URMITE), Aix-Marseille University, Marseille, France, and Microbial Ecology laboratory, Béjaia University, Béjaia, Algeria (Table 1). Carbapenemase activity was assessed using phenotypic and genotypic tests, including the modified Hodge test, MALDI-TOF MS assay, PCR amplification and sequencing [4,11].

The Carba NP and the CarbAcineto NP tests are straightforward biochemical tests which identify carbapenemase production in GNB by detecting imipenem hydrolysis using phenol red solution as a colour indicator and a bacterial lysis buffer (B-PER II, Bacterial Protein Extraction Reagent) for *Enterobacteriaceae* and *Pseudomonas* species (Carba NP test) [5,6] and 5 M NaCl for *Acinetobacter* species (CarbAcineto NP test) [7].

In order to use a single protocol to detect the production of carbapenemases in the three types of bacteria

http://dx.doi.org/10.1016/j.nmni.2015.07.001

TABLE 1. Test results for *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains

Group	Species	Carbapenemase or other β-lactamase gene	Test result by:		
			MHT	**MALDI-TOF MS**	**MCNP**
Enterobacteriaceae	*Klebsiella pneumoniae* H5500[M]	*NDM-1*	+	+	+
	K. pneumoniae Kpnasey[M]	*NDM-1*	+	+	+
	K. pneumoniae U2A 2246[M]	*KPC-3*	+	+	+
	K. pneumoniae[A]	*KPC-3*	+	+	+
	K. pneumoniae 360[M]	*KPC-2*	+	+	+
	K. pneumoniae[M]	*OXA-48*	+	+	+
	K. pneumoniae 413[A]	*TEM-1/CTX-M-3/SHV-12/DHA-1*	–	–	–
	K. pneumoniae 473[A]	*TEM-1/CTX-M-15/OXA-1*	–	–	–
	K. pneumoniae 463[A]	*TEM-1/CTX-M-15*	–	–	–
	K. pneumoniae 550[A]	*TEM-1/CTX-M-3*	–	–	–
	K. pneumoniae 47[A]	*TEM-1/CMY-4*	–	–	–
	K. pneumoniae 123[A]	*TEM-1/CMY-4*	–	–	–
	K. pneumoniae 318[A]	*CTX-M-15*	–	–	–
	K. pneumoniae 476[A]	*CTX-M-15*	–	–	–
	K. pneumoniae 613[A]	*CMY-4*	–	–	–
	K. pneumoniae 615[A]	*CMY-4*	–	–	–
	Escherichia coli H5636[M]	*NDM-1*	+	+	+
	E. coli 181[A]	*NDM-5*	+	+	+
	E. coli[M]	*NDM-5*	+	+	+
	E. coli 99[A]	*OXA-48*	+	+	+
	E. coli 100[A]	*OXA-48*	+	+	+
	E. coli 132[A]	*OXA-48*	+	+	+
	E. coli 192[A]	*OXA-48*	+	+	+
	E. coli[M]	*OXA-48*	+	+	+
	E. coli 544[A]	*TEM-1/CTX-M-15/OXA-1*	–	–	–
	E. coli 469[A]	*TEM-1/CTX-M-3/OXA-1*	–	–	–
	E. coli 472[A]	*TEM-1/CTX-M-3/OXA-1*	–	–	–
	E. coli 234[A]	*TEM-1/CTX-M-15/CMY-4*	–	–	–
	E. coli 611[A]	*TEM-1/CTX-M-15/CMY-4*	–	–	–
	E. coli 536[A]	*TEM-1/CTX-M-15*	–	–	–
	E. coli 534[A]	*CTX-M-15/OXA-1*	–	–	–
	E. coli 542[A]	*CTX-M-15/OXA-1*	–	–	–
	E. coli 560[A]	*TEM-1/CMY-4*	–	–	–
	E. coli 606[A]	*TEM-1/CMY-4*	–	–	–
	E. coli ATCC 25922[M]	—	–	–	–
	E. coli 7624[M]	—	–	–	–
	E. cloacae 308[A]	*TEM-1/CTX-M-15/OXA-1*	–	–	–
	E. cloacae 570[A]	*TEM-1/CTX-M-15*	–	–	–
Pseudomonas	*P. aeruginosa*[M]	*VIM-2*	+	+	+
	P. aeruginosa[M]	*VIM-2*	+	+	+
	P. aeruginosa[M]	*VIM-2*	+	+	+
	P. aeruginosa[M]	*VIM-2*	+	+	+
	P. aeruginosa[M]	*VIM-2*	+	+	+
	P. aeruginosa[M]	*VIM-2*	+	+	+
	P. aeruginosa[M]	*VIM-2*	+	+	+
	P. aeruginosa[M]	*VIM-2*	+	+	+
	P. aeruginosa[M]	*VIM-2*	+	+	+
	P. aeruginosa[M]	*VIM-2*	+	+	+
	P. aeruginosa[M]	—	–	–	–
	P. aeruginosa[M]	—	–	–	–
	P. aeruginosa[M]	—	–	–	–
	P. aeruginosa[M]	—	–	–	–
	P. aeruginosa[M]	—	–	–	–
	P. aeruginosa UAA 2257[M]	*IMP-1*	+	+	+
Acinetobacter	*A. baumannii*[A]	*OXA-23*	+	+	+
	A. baumannii[A]	*OXA-23*	+	+	+
	A. baumannii[A]	*OXA-23*	+	+	+
	A. baumannii[A]	*OXA-23*	+	+	+
	A. baumannii[A]	*OXA-23*	+	+	+
	A. baumannii[A]	*OXA-23*	+	+	+
	A. baumannii[M]	*OXA-23*	+	+	+
	A. baumannii[M]	*OXA-23*	+	+	+
	A. baumannii[M]	*OXA-23*	+	+	+
	A. baumannii[M]	*OXA-23*	+	+	+
	A. baumannii[M]	*OXA-24*	+	+	+
	A. baumannii[M]	*OXA-24*	+	+	+
	A. baumannii[M]	*OXA-24*	+	+	+
	A. baumannii[M]	*OXA-24*	+	+	+
	A. baumannii[M]	*OXA-24*	+	+	+
	A. baumannii[M]	*OXA-24*	+	+	+
	A. baumannii[A]	*OXA-24*	+	+	+
	A. baumannii[A]	*OXA-24*	+	+	+
	A. baumannii[A]	*OXA-24*	+	+	+
	A. baumannii[A]	*OXA-24*	+	+	+
	A. baumannii[M]	*OXA-58*	+	+	+
	A. baumannii[M]	*OXA-23/OXA-24*	+	+	+
	A. baumannii[A]	*OXA-23/OXA-24*	+	+	+
	A. baumannii[A]	*OXA-23/OXA-24*	+	+	+
	A. baumannii[A]	*NDM-1*	+	+	+
	A. baumannii[A]	*NDM-1*	+	+	+
	A. baumannii[A]	*NDM-1*	+	+	+
	A. baumannii[A]	*NDM-1*	+	+	+
	A. baumannii[A]	*NDM-1*	+	+	+
	A. baumannii[M]	*NDM-1*	+	+	+

TABLE 1. Continued

Group	Species	Carbapenemase or other β-lactamase gene	Test result by: MHT	MALDI-TOF MS	MCNP
	A. baumannii[M]	*NDM-1*	+	+	+
	A. baumannii[M]	*NDM-1*	+	+	+
	A. baumannii[M]	*NDM-1*	+	+	+
	A. baumannii[M]	*NDM-1*	+	+	+
	A. baumannii[M]	*OXA-23/NDM-1*	+	+	+
	A. baumannii[M]	*OXA-23/NDM-1*	+	+	+
	A. baumannii[M]	*OXA-23/NDM-1*	+	+	+
	A. baumannii[M]	*OXA-23/NDM-1*	+	+	+
	A. baumannii[M]	*OXA-23/NDM-1*	+	+	+
	A. baumannii[M]	*OXA-23/NDM-1*	+	+	+
	A. baumannii[A]	*OXA-23/NDM-1*	+	+	+
	A. baumannii[A]	*OXA-23/NDM-1*	+	+	+
	A. baumannii[A]	*OXA-23/NDM-1*	+	+	+
	A. baumannii[A]	*OXA-23/NDM-1*	+	+	+
	A. baumannii[A]	*TEM-128*	−	−	−
	A. baumannii[A]	*TEM-128*	−	−	−
	A. baumannii[A]	*TEM-128*	−	−	−
	A. baumannii[A]	*TEM-128*	−	−	−
	A. baumannii[A]	*TEM-128*	−	−	−
	A. baumannii[A]	*TEM-128*	−	−	−
	A. baumannii[A]	*TEM-128*	−	−	−
	A. baumannii[M]	*TEM-128*	−	−	−
	A. baumannii[M]	*TEM-128*	−	−	−
	A. baumannii[M]	*TEM-128*	−	−	−
	A. baumannii AYE[M]	*VEB-1*	−	−	−
	A. baumannii SDF[M]	—	−	−	−

MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MCNP, modified Carba NP test; MHT, modified Hodge test.
[A]Strains tested in Microbial Ecology Laboratory, Béjaia University, Béjaia, Algeria.
[M]Strains tested in Unité de recherche sur les maladies infectieuses et tropicales émergentes (URMITE), Aix-Marseille University, Marseille, France.

(*Enterobacteriaceae, Pseudomonas* and *Acinetobacter*) and to accelerate the speed with which results are produced, the lysis buffer and pH of the colour indicator solution used in the Carba NP and CarbAcineto NP tests were changed.

In the MCNP test, the lysis buffers used for the Carba NP test and CarbAcineto NP test, B-PER II, Bacterial Protein Extraction Reagent and NaCl 5 M, respectively, were replaced by cetyl trimethyl ammonium bromide (CTAB) 0.02%, and the pH value of the phenol red solution was adjusted to 7.5 (instead of 7.8). In addition, two steps used in the previous protocols [5,6], centrifugation and incubation at room temperature for 30 minutes, were eliminated in our method. These modifications simplify the lysis step and produce results more quickly.

The MCNP test was performed as follows. One inoculation loop (10 µL) of the tested strain, directly recovered from a Mueller Hinton agar plate (bioMérieux, Marcy l'Étoile, France), was resuspended in 200 µL of 0.02% CTAB (Sigma-Aldrich Chimie, Saint-Quentin-Fallavier, France) and vortexed for 1 to 2 minutes. Subsequently, 100 µL of the bacterial suspension was mixed with 100 µL of diluted phenol red solution (2 mL of phenol red (Sigma-Aldrich) solution 0.5% (wt/vol) with 16.6 mL of distilled water) containing 0.1 mM $ZnSO_4$ (pH 7.5) in the first tube, tube 1, used as negative control, and a diluted phenol red

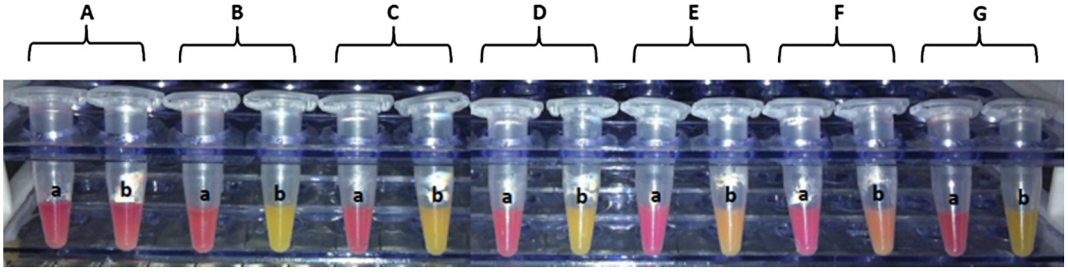

FIG. 1. Modified Carba NP test results for *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* species. (A) *Escherichia coli* ATCC 25922. (B) *NDM-5*-positive *E. coli*. (C) *KPC-2*-positive *Klebsiella pneumoniae* 360. (D) *IMP-1*-positive *Pseudomonas aeruginosa* UAA 2257. (E) *OXA-23*-positive *Acinetobacter baumannii*. (F) *OXA-24*-positive *A. baumannii*. (G) *NDM-1*-positive *A. baumannii*. (a) Tube containing phenol red solution 0.1 mM $ZnSO_4$ (pH 7.5) and cetyl trimethyl ammonium bromide (CTAB) 0.02%. (b) Tube containing phenol red solution 0.1 mM $ZnSO_4$ (pH 7.5) supplemented with 6 mg/mL of imipenem and CTAB 0.02%.

solution containing 0.1 mM $ZnSO_4$ (pH 7.5) supplemented with 6 mg/mL of commercially available imipenem (Tienam 500; Merck Sharp & Dohme, Paris, France) in the second tube, tube 2. Tubes 1 and 2 were vortexed, then incubated at 37°C for a maximum of 2 hours.

Carbapenemase activity was revealed when the test and negative control solutions, respectively, were yellow vs. red or orange vs. red. In contrast, both solutions remained red in the case of noncarbapenemase producers (Fig. 1).

The results showed that the MCNP method detected all carbapenemases produced by carbapenem-resistant strains with 100% sensitivity and 100% specificity. Positive results were observed at different times for the different carbapenemases types (MBL, KPC and OXA-48 at 10 to 30 minutes vs. 1 to 2 hours for OXA type). The most interesting aspect of this method is that the colour changed from red to orange or yellow (positive result) even before incubation in some cases (*NDM-5*-producing *Escherichia coli, NDM-1*-producing *Klebsiella pneumoniae* (Kpnasey) and imipenem-producing *P. aeruginosa* UAA2257). Moreover, a higher inoculums (two inoculation loops (10 µL)) is recommended for *Acinetobacter* species tests.

Currently, the MCNP test is routinely used in Timone Hospital, Marseille, France. It was performed when antibiotic susceptibility testing revealed a resistance to ertapenem and susceptibility or resistance to imipenem. The suspicion of carbapenemase producers, in particular OXA-48, was based on this phenotype. Between November 2014 and May 2015, a total of 233 strains were tested. Among them, 35 positives strains with carbapenemase producers were detected (Table 2). These positives strains were isolated from 25 different patients. These results confirm the efficiency of the MCNP test with high sensitivity, given the detection of all strains producing OXA-48-type carbapenemases. Also, two carbapenemase-producing *A. baumannii* were detected, thus confirming the advantages of the MCNP test.

In conclusion, the advantages of the MCNP test are the detection of different carbapenemase types from *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* species using a single protocol, as well as the short time to results, particularly in the case of MBL-producing *Enterobacteriaceae* and *Pseudomonas* species. In addition, the effectiveness of this test on a large series of bacteria may allow us to identify the production

TABLE 2. Results of MCNP test applied for carbapenem-resistant strains isolated in La Timone Hospital, Marseille, France

			Antibiotic susceptibility testing results			
Date	**Sample source**	**Strain**	**ETP**	**IMP**	**MCNP result**	**Carbapenemases gene detected**
10/11/2014	Urine	*Klebsiella pneumoniae*	R	R	+	*OXA-48*
20/11/2014	Rectal swab	*Escherichia coli*	R	S	+	*OXA-48*
25/11/2014	Bronchoalveolar lavage fluid	*K. pneumoniae*	R	S	+	*OXA-48*
05/12/2014	Bronchoalveolar lavage fluid	*K. pneumoniae*	R	R	+	*OXA-48*
08/12/2014	Rectal swab	*K. pneumoniae*	R	S	+	*OXA-48*
09/12/2014	Blood culture	*K. pneumoniae*	R	R	+	*OXA-48*
11/12/2014	Urine	*Enterobacter cloacae*	R	S	+	*OXA-48*
21/12/2014	Stools	*K. pneumoniae*	R	S	+	*OXA-48*
31/12/2014	Spittle	*K. pneumoniae*	R	R	+	*OXA-48*
12/01/2015	Urine	*E. coli*	R	S	+	*OXA-48*
24/01/2015	Armpit swab	*K. pneumoniae*	R	S	+	*OXA-48*
02/02/2015	Rectal swab	*K. pneumoniae*	R	R	+	*NDM*
06/02/2015	Urine	*E. coli*	R	S	+	*OXA-48*
17/02/2015	Urine	*E. coli*	R	S	+	*OXA-48*
19/02/2015	Spittle	*K. pneumoniae*	R	S	+	*OXA-48*
20/02/2015	Rectal swab	*K. pneumoniae*	R	S	+	*OXA-48*
23/02/2015	Urine	*K. pneumoniae*	R	S	+	*OXA-48*
04/03/2015	Rectal swab	*E. cloacae*	R	R	+	*OXA-48*
04/03/2015	Rectal swab	*K. pneumoniae*	R	R	+	*OXA-48*
16/03/2015	Rectal swab	*K. pneumoniae*	R	S	+	*OXA-48*
23/03/2015	Bronchial aspirate	*K. pneumoniae*	R	S	+	*OXA-48*
30/03/2015	Armpit swab	*K. pneumoniae*	R	S	+	*OXA-48*
31/03/2015	Urine	*K. pneumoniae*	R	S	+	*OXA-48*
13/04/2015	Sinus	*Serratia marcescens*	R	R	+	*OXA-48*
13/04/2015	Blood culture	*E. cloacae*	R	S	+	*OXA-48*
18/04/2015	Blood culture	*E. coli*	R	I	+	*OXA-48*
18/04/2015	Rectal swab	*K. pneumoniae*	R	S	+	*OXA-48*
18/04/2015	Blood culture	*E. coli*	R	I	+	*OXA-48*
04/05/2015	Urine	*K. pneumoniae*	R	R	+	*OXA-48*
30/04/2015	Urine	*K. pneumoniae*	R	I	+	*NDM-1*
07/05/2015	Bronchial aspirate	*K. pneumoniae*	R	I	+	*OXA-48*
08/05/2015	Blood culture	*Acinetobacter baumannii*	NT	R	+	*OXA-23*
11/05/2015	Urine	*A. baumannii*	NT	R	+	*OXA-23*
18/05/2015	Rectal swab	*K. pneumoniae*	R	I	+	*OXA-48*

ETP, ertapenem; IMP, imipenem; MCNP, modified Carba NP test; NT, not tested; R, resistant; S, susceptible; I, Intermediate.

of carbapenemase enzymes even before identification of the bacterial strain.

Interestingly, as well as using this test in developed countries such as France (URMITE laboratories, La Timone Hospital), given the simplicity and the low cost of the MCNP test, it could be used by any laboratory, including laboratories in developing countries. In Algeria, this test has been used in the Microbial Ecology Laboratory of Béjaia University since May 2014, and it will soon be introduced to laboratories in Algerian hospitals.

Conflict of interest

None declared.

Acknowledgements

We thank L. Hadjadj, A. Bergal, M. A. El-Gawad El-Sayed and N. Mathlouthi for their contributions. Supported in part by CNRS and IHU Méditerranée Infection.

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