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*	+	+	+