Published in "Journal of Medical Microbiology
doi: 10.1099/jmm.0.071340-0, 2014"
which should be cited to refer to this work.

Impact of the isolation medium for detection of carbapenemase-producing *Enterobacteriaceae* using an updated version of the Carba NP test

Carbapenem resistance in *Enterobacteriaceae* is now emerging worldwide at an alarming rate, causing both nosocomial and now community-acquired infections (Nordmann *et al.*, 2012a). A variety of carbapenemases have been reported in *Enterobacteriaceae* such as KPC (Ambler class A), metallo-β-lactamases of VIM-, IMP- and NDM-type (Ambler class B), and OXA-48-types (Ambler class D). Thus, an efficient strategy for detection of carbapenemase producers is becoming critical for the determination of appropriate therapeutic schemes and the implementation of infection control measures (Nordmann & Poirel, 2013). Recently, the Carba NP test has been developed for rapid identification of carbapenemase production in *Enterobacteriaceae* (Nordmann *et al.*, 2012b). Here, we further improve and evaluate the ability of the Carba NP test to detect carbapenemase producers among *Enterobacteriaceae* recovered from various commercial media (selective, non-selective and screening media) used in clinical situations.

Fifty carbapenemase-producers (10 OXA-48, 10 KPC, 10 VIM, 10 NDM and 10 IMP) were included in the study (Table 1). Twelve non-carbapenemase-producing enterobacterial strains non-susceptible to carbapenems were also included. All strains had previously been characterized for their β-lactamase content at the molecular level (Nordmann *et al.*, 2012b). Susceptibilities to carbapenems were determined by Etest (bioMérieux) and interpreted according to EUCAST breakpoints (www.eucast.org/clinical_breakpoints/), as updated in 2013.

Prior to performing the Carba NP test, all strains were grown at 37 °C for 24 h on the following media.

(i) Non-selective media were Columbia agar + 5 % sheep blood (bioMérieux), Tryticase soy agar (bioMérieux) and the non-selective chromogenic medium UriSelect 4 (Bio-Rad).

(ii) Gram-negative bacilli (GNB) selective media were Drigalski agar (Bio-Rad) and MacConkey agar (Becton Dickinson).

(iii) Screening media for screening extended-spectrum β lactamase (ESBL) or carbapenemase producers were ChromID ESBL (bioMérieux), Brilliance CRE Agar (Oxoid) and CHROMagar KPC (CHROMagar).

(iv) Mueller–Hinton (MH) agar from bioMérieux (Mueller–Hinton 2 agar), Bio-Rad (Mueller–Hinton agar), Oxoid (CM0337 Mueller–Hinton agar) and Becton Dickinson (BBL Mueller–Hinton II agar) were prepared as recommended by the manufacturers. MH agar supplemented with $ZnSO_4$ was prepared by diluting a concentrated sterile solution of $ZnSO_4$ (Merck Millipore) to a final concentration of 70 μg ml^{-1}.

The Carba NP test is based on the colorimetric detection of hydrolysis of the β-lactam ring of a carbapenem molecule, imipenem (Nordmann *et al.*, 2012b). We have further improved the Carba NP test by using 1.5 ml Eppendorf tubes instead of 96-well microplates and by using a reduced amount of bacteria (1/4 to 1/3 of a calibrated 10 μl loop resuspended in 100 μl of lysis buffer). These modifications simplify the lysis step for obtaining the bacterial extracts, eliminating the need for a centrifugation step (see methods available in the online Supplementary Material).

Our results confirmed the excellent sensitivity (100 %) and specificity (100 %) of the Carba NP test when bacterial isolates were recovered on non-selective rich media (i.e. Columbia blood agar and trypticase soy agar) (Table 1) (Nordmann *et al.*, 2012b).

Using the non-selective chromogenic medium UriSelect 4, all carbapenemase producers were detected (Table 1), indicating that chromogenic molecules used in this medium to differentiate between enterobacterial species did not interfere with the Carba NP test.

Considering Gram-negative selective media, regardless of carbapenemase production, the Carba NP test was interpretable for only 63 % and 71 % of the strains recovered from Drigalski agar and MacConkey agar, respectively (Table 1). In addition, when the Carba NP test was interpretable, OXA-48, KPC, VIM and IMP producers were all detected, whereas only 50 % of NDM producers were detected.

The Carba NP test was previously validated on Becton Dickinson MH agar (Nordmann *et al.*, 2012b). However, it is known that commercially available MH agar media differ in their ion content. Consequently, the Carba NP test was performed on colonies obtained from MH agar media from four different manufacturers. Whatever the medium used, 100 % of OXA-48, KPC and IMP producers were detected. However, detection of all VIM and NDM producers was obtained only with MH agars from Becton Dickinson and Oxoid. The Carba NP test detected only 10 % of NDM producers and 30 % of VIM producers grown on bioMérieux MH agar. Intermediate results were obtained with strains grown on Bio-Rad MH agar, since all VIM producers but only 30 % of NDM producers were detected. Since detection failures concerned only metallo-β-lactamase producers, which are known to use zinc ions in their catalytic site, the ability to detect carbapenemase production from isolates recovered from the four MH agars may be related to their zinc concentration. Accordingly, all carbapenemase producers were detected

Table 1. Detection of carbapenemase and non-carbapenemase producers from strains isolated on various media using the Carba NP test (+, positive result; −, negative result; NI, not interpretable; 0, no growth)

Species	β-Lactamase content		MIC* (μg ml^{-1})			Non-selective media			GNB selective media		Mueller–Hinton								Screening media		
	Carbapenemase	ESBLs/cephalosporinases†	IMP	MER	ERT	Blood agar	Tripticase soy agar	UriSelect 4	McConkey	Drigalski	bioMérieux	bioMérieux + Zn^{2+}	Bio-Rad	Bio-Rad + Zn^{2+}	Oxoid	Oxoid + Zn^{2+}	Becton Dickinson	Becton Dickinson + Zn^{2+}	ChromID^{TM} ESBL	Brilliance CRE Agar	CHROMagar KPC
Escherichia coli ROV	OXA-48	None	0.5	0.25	0.75	+	+	+	+	NI	+	+	+	+	+	+	+	+	0	0	0
E. coli OMA 22	OXA-48	CTX-M-15	0.5	0.25	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
Klebsiella pneumoniae BIC	OXA-48	None	0.5	0.5	2	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0
K. pneumoniae CHA	OXA-48	None	0.38	0.5	1	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0
K. pneumoniae EGY	OXA-48	CTX-M-15	2	2	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
K. pneumoniae BEL	OXA-48	None	1	1	4	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0
K. pneumoniae RAM	OXA-48	None	1	1	4	+	+	+	NI	NI	+	+	+	+	+	+	+	+	0	+	0
K. pneumoniae DIAR	OXA-48	CTX-M-15	>32	>32	>32	+	+	+	NI	NI	+	+	+	+	+	+	+	+	+	+	+
Enterobacter cloacae BEU	OXA-48	CTX-M-15 + CTX-M-12	0.5	0.5	8	+	+	+	NI	+	+	+	+	+	+	+	+	+	+	+	0
Citrobacter koseri ROU	OXA-48	None	0.38	0.38	2	+	+	+	NI	NI	+	+	+	+	+	+	+	+	0	0	0
E. coli LIL-1	KPC-2	None	2	1	1.5	+	+	+	NI	NI	+	+	+	+	+	+	+	+	+	+	+
E. coli PSP	KPC-2	None	0.5	0.5	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
K. pneumoniae KN 633	KPC-2	CTX-M-12	>32	4	>32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae A28006	KPC-2	CTX-M-2	16	32	24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae POZ	KPC-3	None	8	2	12	+	+	+	+	NI	+	+	+	+	+	+	+	+	+	+	+
E cloacae HGM 83048	KPC-2	None	24	16	>32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. cloacae HPTU 27040557	KPC-2	None	1	0.75	1.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
Citrobacter freundii HPTU	KPC-2	None	8	3	1.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serratia marcecens CGNC 7072	KPC-2	SHV-12	>32	>32	>32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. marcecens D 6403	KPC-2	None	>32	>32	>32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. coli 271	NDM-1	CTX-M-15	6	16	32	+	+	+	+	NI	−	+	−	+	+	+	+	+	+	+	+
E. coli ALL	NDM-1	CTX-M-15	4	8	>32	+	+	+	−	NI	−	+	−	+	+	+	+	+	+	+	+
E. coli FEK	NDM-4	CTX-M-15	>32	>32	>32	+	+	+	−	NI	−	+	+	+	+	+	+	+	+	+	+
K. pneumoniae 601	NDM-1	CTX-M-15 + SHV-28	>32	>32	>32	+	+	+	NI	NI	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae 419	NDM-1		1.5	2	6	+	+	+	NI	NI	−	+	+	+	+	+	+	+	+	+	+

Species	β-Lactamase content		MIC* (μg ml^{-1})			Non-selective media			GNB selective media		Mueller–Hinton								Screening media		
	Carbapenemase	ESBLs/cephalosporinases†	IMP	MER	ERT	Blood agar	Tripticase soy agar	UriSelect 4	McConkey	Drigalski	bioMérieux	bioMérieux + Zn^{2+}	Bio-Rad	Bio-Rad + Zn^{2+}	Oxoid	Oxoid + Zn^{2+}	Becton Dickinson	Becton Dickinson + Zn^{2+}	ChromID^{TM} ESBL	Brilliance CRE Agar	CHROMagar KPC
K. pneumoniae AFR7	NDM-1	CTX-M-15 + CMY-6 + SHV-28	>32	>32	>32	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+
K. pneumoniae DIN	NDM-1	CTX-M-15 + CMY-6	1	3	16	+	+	+	−	−	−	+	−	+	+	+	+	+	+	+	+
K. pneumoniae KIE	NDM-1	SHV-38 + CMY-16	0.75	1	2	+	+	+	+	**NI**	−	+	−	+	−	+	+	+	+	+	**0**
Providencia rettgeri IR38	NDM-1	NDM-1 + CTX-M-15	3	1.5	0.5	+	+	+	**NI**	**NI**	−	+	−	+	+	+	+	+	+	+	+
Salmonella CAN	NDM-1	CTX-M-15				+	+	+	**NI**	**NI**	−	+	−	+	+	+	+	+	+	+	+
E. coli MAD	VIM-1	CTX-M-3	1.5	0.5	0.38	+	+	+	**NI**	**NI**	−	+	+	+	−	+	+	+	+	+	**0**
K. pneumoniae DIH	VIM-19	CTX-M-3	8	4	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**0**	+
K. pneumoniae MAD	VIM-1	CTX-M-3	1	1	0.5	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	**0**
K. pneumoniae ENN	VIM-1	SHV-5	0.5	0.38	4	+	+	+	**NI**	**NI**	−	+	+	+	+	+	+	+	+	+	**0**
K. pneumoniae HAS	VIM-4	SHV-12 + CMY-4	2	2	1	+	+	+	**NI**	**NI**	−	+	+	+	+	+	+	+	+	+	+
K. pneumoniae ROU	VIM-1	CTX-M-15	1.5	0.75	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. cloacae KAR	VIM-1	SHV-70	1	0.5	0.38	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**0**	**0**
E. cloacae KOW	VIM-4	CTX-M-15 + SHV-31	4	3	2	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
C. freundii CAT	VIM-2	None	1.5	0.75	2	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
C. freundii MIG	VIM-2	None	1.5	0.5	4	+	+	+	**NI**	**NI**	−	+	+	+	+	+	+	+	+	+	+
E. coli JAP	IMP-1	None	0.5	0.5	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**0**	**0**
E. coli TWA	IMP-8	SHV-12	6	3	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K. pneumoniae 6852	IMP-1	SHV-5	1	8	2	+	+	+	**NI**	**NI**	+	+	+	+	+	+	+	+	+	+	**0**
K. pneumoniae 1 TWA	IMP-8	None	1	0.5	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**0**
K. pneumoniae 2 TWA	IMP-8	SHV −12	0.5	0.5	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**0**
K. pneumoniae BM1	IMP-1	None	1.5	1	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**0**	+
K. pneumoniae BM2	IMP-1	TEM-15	8	2	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**0**	**0**
K. pneumoniae BM3	IMP-1	TEM-15 + CTX-M-15	1.5	2	4	+	+	+	**NI**	**NI**	+	+	+	+	+	+	+	+	+	**0**	+
E. cloacae 1 TWA	IMP-8	None	1.5	1	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. cloacae 2 TWA	IMP-8	SHV-12	0.75	0.5	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. coli MAR	None	Overexpressed chromosomal case	16	2	>32	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
K. pneumoniae FOS	None	CTX-M-15	6	>32	>32	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Table 1. cont.

Species	β-Lactamase content		MIC* (μg ml^{-1})			Non-selective media			GNB selective media		Mueller–Hinton								Screening media		
	Carbapenemase	ESBLs/cephalosporinases†	IMP	MER	ERT	Blood agar	Tripticase soy agar	UriSelect 4	McConkey	Drigalski	bioMérieux	bioMérieux + Zn^{2+}	Bio-Rad	Bio-Rad + Zn^{2+}	Oxoid	Oxoid + Zn^{2+}	Becton Dickinson	Becton Dickinson + Zn^{2+}	ChromID™ ESBL	Brilliance CRE Agar	CHROMagar KPC
K. pneumoniae ANG	None	DHA-1	>32	>32	>32	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
K. pneumoniae COO	None	CTX-M-15 + SHV-28	8	4	>32	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
K. pneumoniae BER	None	SHV-28	1	1	4	–	–	–	**NI**	**NI**	–	–	–	–	–	–	–	–	–	–	–
K. pneumoniae SCH	None	CTX-M-15	3	3	>32	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E. cloacae HUR	None	Overexpressed chromosomal Case	0.19	0.19	1.5	–	–	–	**NI**	**NI**	–	–	–	–	–	–	–	–	–	–	–
E. cloacae POG	None	Overexpressed chromosomal Case	4	3	1.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**0**	–
E. cloacae MEU	None	Overexpressed chromosomal Case	6	4	>32	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**0**	–
Enterobacter aerogenes DEL	None	Overexpressed chromosomal Case	1	0.75	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Morganella morgannii MAU	None	Overexpressed chromosomal Case	2	0.19	0.023	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
M. morgannii DAU	None	Overexpressed chromosomal Case	1.5	0.19	0.016	–	–	–	**NI**	**NI**	–	–	–	–	–	–	–	–	–	–	–

*MIC, minimum inhibitory concentration; IMP, imipenem; MER, meropenem; ERT, ertapenem.
†Case, cephalosporinase.

using the Carba NP test when bacterial isolates were recovered from zinc-supplemented MH agar, regardless of the manufacturer.

Since the prevention of carbapenemase-producing *Enterobacteriaceae* dissemination relies on accurate detection of colonized patients, three screening media were evaluated (CHROMagar KPC, Brilliance CRE and ChromID ESBL). As soon as the bacterial isolates were able to grow on those selective media, the Carba NP test differentiated perfectly between carbapenemase and non-carbapenemase producers (Table 1).

The Carba NP test was recently demonstrated to be of great value for excellent and rapid identification of carbapenemase producers (of any type) (Milillo *et al.*, 2013; Nordmann *et al.*, 2012b; Tijet *et al.*, 2013; Vasoo *et al.*, 2013). However, these data were obtained with bacterial isolates grown on only one type of MH agar (Becton Dickinson). In this study, we demonstrated that the Carba NP test might be performed on rich media in current use to isolate clinical samples, such as blood agar and trypticase soy agar. Moreover, when considering urine specimens, chromogenic non-selective media, such as UriSelect 4 medium, are often used in clinical microbiology to provide presumptive identification of the bacterial species responsible for urinary tract infections (UTIs). In the case of UTIs caused by a carbapenemase-producing isolate, our results showed that the Carba NP test might be performed directly on the chromogenic medium with 100 % sensitivity and specificity. These results are of utmost importance, especially in endemic situations of carbapenemase producers (Nordmann *et al.*, 2012a).

Our results indicate that the zinc concentration in MH agar of the medium is crucial for efficient detection of VIM and NDM-type carbapenemases. These results are similar to those obtained with the modified Hodge test, for which higher sensitivity can be obtained using zinc-supplemented plates. However, with the Carba NP test, the IMP enzymes were always detected regardless of the zinc content of the MH agar used. The higher catalytic activity of IMP-1 compared with NDM-1 and VIM-2 (Yong *et al.*, 2009), coupled with its active site plasticity, might explain these results (Horton *et al.*, 2012).

Finally, 30 % of the strains gave non-interpretable results when bacterial colonies were recovered from Drigalski or MacConkey agar, regardless of carbapenemase production. Such results might be a consequence of the accumulation of lactic acid in the bacterial isolates that were able to ferment lactose. Therefore, the Carba NP test is not suitable to identify carbapenemase producers grown on Drigalski agar and MacConkey agar plates.

Transparency declaration

An international patent form for the Carba NP test has been filed on behalf of INSERM Transfert (Paris, France).

Acknowledgements

This work was partially funded by a grant from the INSERM (UMR914), by the Université Paris-Sud, France and by grants from the European Community (R-GNOSIS, FP7/HEALTH-F3-2011-282512 and MAGIC-BULLET, FP7/HEALTH-F3-2001-278232).

Laurent Dortet,[1] Ludivine Bréchard,[1] Laurent Poirel[1,2] and Patrice Nordmann[1,2]

[1]INSERM U914 'Emerging Resistance to Antibiotics', Le Kremlin-Bicêtre, France

[2]Medical and Molecular Microbiology, Department of Medicine, Faculty of Science, University of Fribourg, Switzerland

Correspondence: Patrice Nordmann (patrice.nordmann@unifr.ch)

Supplementary methods are available with the online version of this paper.

Horton, L. B., Shanker, S., Mikulski, R., Brown, N. G., Phillips, K. J., Lykissa, E., Venkataram Prasad, B. V. & Palzkill, T. (2012). Mutagenesis of zinc ligand residue Cys221 reveals plasticity in the IMP-1 metallo-β-lactamase active site. *Antimicrob Agents Chemother* **56**, 5667–5677.

Milillo, M., Kwak, Y. I., Snesrud, E., Waterman, P. E., Lesho, E. & McGann, P. (2013). Rapid and simultaneous detection of bla_{KPC} and bla_{NDM} by use of multiplex real-time PCR. *J Clin Microbiol* **51**, 1247–1249.

Nordmann, P. & Poirel, L. (2013). Strategies for identification of carbapenemase-producing *Enterobacteriaceae*. *J Antimicrob Chemother* **68**, 487–489.

Nordmann, P., Dortet, L. & Poirel, L. (2012a). Carbapenem resistance in *Enterobacteriaceae*: here is the storm! *Trends Mol Med* **18**, 263–272.

Nordmann, P., Poirel, L. & Dortet, L. (2012b). Rapid detection of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* **18**, 1503–1507.

Tijet, N., Boyd, D., Patel, S. N., Mulvey, M. R. & Melano, R. G. (2013). Evaluation of the Carba NP test for rapid detection of carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* **57**, 4578–4580.

Vasoo, S., Cunningham, S. A., Kohner, P. C., Simner, P. J., Mandrekar, J. N., Lolans, K., Hayden, M. K. & Patel, R. (2013). Comparison of a novel, rapid chromogenic biochemical assay, the Carba NP test, with the modified Hodge test for detection of carbapenemase-producing Gram-negative bacilli. *J Clin Microbiol* **51**, 3097–3101.

Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K. & Walsh, T. R. (2009). Characterization of a new metallo-β-lactamase gene, $bla_{(NDM-1)}$, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother* **53**, 5046–5054.