Performance of a new in-house medium Carba MTL-broth for the rapid detection of carbapenemase-producing Enterobacteriaceae

Assia Mairi, Abdelaziz Touati, Alix Pantel, Catherine Dunyach-Remy, Albert Sotto, Christophe de Champs, Jean-Philippe Lavigne

JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Performance of a new in-house medium Carba MTL-broth for the rapid detection of carbapenemase-producing *Enterobacteriaceae*

Assia Mairi[1,2], Abdelaziz Touati[1], Alix Pantel[3], Catherine Dunyach-Remy[3], Albert Sotto[4], Christophe De Champs[5,6], Jean-Philippe Lavigne[3]

[1] Laboratoire d'Ecologie Microbienne, FSNV, Université de Bejaia, Bejaia, Algeria
[2] Institut National de la Santé et de la Recherche Médicale, U1047, Université de Montpellier, UFR de Médecine, Nîmes, France
[3] U1047, INSERM, Université de Montpellier, Service de Microbiologie, CHU Nîmes, Nîmes, France
[4] U1047, INSERM, Université de Montpellier, Service des Maladies Infectieuses et Tropicales, CHU Nîmes, Nîmes, France
[5] Laboratoire de Bactériologie-Virologie-Hygiène, CHU Reims, Hôpital Robert Debré, Reims, France
[6] INSERM, UMR-S 1250 P3 Cell, UFR de Médecine, Université de Reims Champagne-Ardenne, Reims, France

Abstract

Introduction: The spread of carbapenemase-producing *Enterobacteriaceae* (CPE) represents a major public health issue. Methods allowing rapid detection of carbapenemases in developing countries are therefore urgently needed. In the current study, we developed a new in-house medium for the rapid detection of CPE isolates, especially OXA-48 producers.

Methodology: A panel of 144 clinical strains previously characterized was tested on in-house Carba MTL-broth medium using four different concentrations of ertapenem (0.5 to 2 mg/L), and compared to chromID® OXA-48 and chromID® CARBA (BioMérieux) media.

Results: Comparative evaluation of the Carba MTL-broth with chromID® OXA-48 and chromID® CARBA showed that chromID® OXA-48 and Carba MTL-broth had the highest sensitivity for detection of OXA-48 producers (93.9% and 100%, respectively) comparatively to chromID® CARBA (21.2%). The chromID® OXA-48 had the highest specificity (100%), as compared to the Carba MTL-broth (65.5%) and chromID® CARBA (84.4%) for the detection of OXA-48 producers.

Conclusions: The in-house Carba MTL-broth developed in this study is sensitive, inexpensive, an easy-to-use phenotypic method for the detection of OXA-48-producing enterobacteria. Given the burden of pan-drug resistance, its implementation in the microbiology laboratory of developing countries could be a useful tool for rapid detection of these bacteria.

Key words: Carbapenemase-producing *Enterobacteriaceae*; carba MTL-broth; developing countries; OXA-48-like; screening medium.

J Infect Dev Ctries 2019; 13(7):591-602. doi:10.3855/jidc.11305

(Received 04 February 2019 – Accepted 17 April 2019)

Introduction

Multidrug resistance is an important Public Health problem and has been increasingly reported throughout the world especially concerning the *Enterobacteriaceae* isolates. One of the most important emerging resistance traits corresponds to the production of carbapenemases hydrolyzing carbapenems, which confer resistance to almost all β-lactams [1,2]. The main groups of carbapenemases identified in *Enterobacteriaceae* are Ambler class A (KPC-type) that are able to hydrolyze all β-lactams except cephamycins, the zinc-dependent metallo-β-lactamases (MBL) Ambler class B (NDM, VIM, and IMP) of hydrolyzing all □-lactams except aztreonam, and the Ambler class D (OXA-48-like) carbapenemases, hydrolyzing carbapenems and broad-spectrum cephalosporins only weakly [3-5]. The level of resistance to carbapenems conferred by those carbapenemase producers may vary significantly, making their detection difficult [6].

Vigilant surveillance, rapid and reliable identification of these strains by the personnel in the clinical microbiology laboratory are essential to effective infection control [7]. In this aim, it is important to define robust standardized screening methods for the detection of carbapenemase-producing *Enterobacteriaceae* (CPEs) which can be used in all laboratories, particularly in developing countries where the carbapenemases are diffused [3,4,8,9]. Currently, different methods are used to detect enzymatic resistance to carbapenems including phenotypic methods (*e.g.* chromogenic media), mass spectrometry, biochemical-based methods (*e.g.* Carba NP test and

Blue-Carba NP test), immunochromatography, carbapenemase inhibitor-based tests and molecular typing (PCR-based detection) [4,10-15]. Some of these methods require specific equipments and substantial expertise and, thus, cannot be implemented in all laboratories, especially in those of developing countries [14].

Several studies have highlighted the potential difficulties in the detection of OXA-48-like producing *Enterobacteriaceae* as such isolates often have low carbapenem MICs and may be inhibited by some selective media that contain carbapenems concentrations which can inhibit these isolates [16]. In Algeria, OXA-48-producing *Enterobacteriaceae* isolates were reported sporadically [8,17]. This situation is probably linked with the missing of bacteriological protocols used routinely in the Algerian laboratories. Indeed, chromogenic media are not available and molecular methods are not yet implemented in this country and are too expensive to be used in the routine. This situation is probably the one that prevails in other developing countries.

The aim of this study was to develop an in-house medium, the Carba MTL-broth to detect CPEs with a specific focus on OXA-48-producers, to examine the analytic and performance parameters of this in-house medium in comparison with commercial chromogenic screening medium chromID® CARBA and chromID® OXA-48 (BioMérieux, Marcy l'Etoile, France).

Methodology

Bacterial collection

A panel of 140 clinical Gram-negative bacilli (GNB) isolates belonging to two collections from the regional multidrug-resistant GNB Reference Lab (the CARB-LR group) in the French Occitanie Region [18,19] and from the laboratory of microbial ecology of the University of Bejaia (Algeria) was tested (Table 1). One hundred thirteen *Enterobacteriaceae* isolates were included with the following distribution: *Escherichia coli* (n = 42), *Klebsiella pneumoniae* (n = 35), *Enterobacter cloacae* (n = 14), *Klebsiella oxytoca* (n = 6), *Citrobacter freundii* (n = 4), *Enterobacter aerogenes* (n = 3), *Proteus mirabilis* (n = 2), *Morganella morganii* (n = 1), *Citrobacter werkmanii* (n = 2), *Citrobacter koseri* (n = 1), *Raoultella ornithinolytica* (n = 1), *Raoultella planticola* (n = 1) and *Pluralibacter gergoviae* (n = 1). Different □-lactam resistance profiles were selected with the following properties: i) resistance to carbapenems (n = 66) involving carbapenemase production (n = 55; 33 OXA-48-like, 10 KPC-type, 7 NDM-type, 4 VIM-type and 1 IMI-type producers) or membrane permeability alterations (n = 11); ii) resistance to third-generation cephalosporins (n = 46) mediated by ESBL (n = 21) or overexpressed/plasmid-mediated cephalosporinases (AmpC, n = 25); iii) susceptible to third-generation cephalosporins (n = 1). Twenty-seven non-fermentative GNB were also included to test the specificity of this medium with: *Pseudomonas aeruginosa* (n = 12), *Acinetobacter baumannii* (n = 12), *Stenotrophomonas maltophilia* (n = 2) and *Acinetobacter nosocomialis* (n = 1). These bacteria presented resistance to carbapenems involving carbapenemase production (n = 20; 8 OXA-23, 4 VIM-type, 2 OXA-24, 2 IMP-1, 2 L1-L2, 1 OXA-58+OXA-23, and 1 NDM-type producers) or membrane permeability alterations (n = 7). Four Gram-positive cocci were included as negative control (*Staphylococcus aureus* ATCC103911, *Staphylococcus aureus* ATCC29213, *Enterococcus faecalis* JH2-2 and *Enterococcus faecalis* V583).

Isolates were previously identified using the VITEK® MS and VITEK® 2 systems (bioMérieux, Marcy l'Etoile, France). Susceptibility to antimicrobial agents was tested by the disc-diffusion method (BioRad, Marnes La Coquette, France) on Mueller–Hinton agar according to recommendations of EUCAST-SFM 2017 (http://www.sfm-microbiologie.org). In addition, the MICs of ertapenem were determined by the Etest® method (bioMérieux, Marcy l'Etoile, France). The MIC of colistin was determined using microbroth dilution (Umic®, Biocentric, Bandol, France). The MICs were interpreted as specified by the EUCAST-SFM criteria. All isolates were typed by the Check-MDR CT102/103® microarrays (Biocentric, Bandol, France). Confirmation of the presence of β-lactamase-encoding genes was done by PCRs using specific primers and confirmed by sequencing the PCR products as previously described [18-22]. Emerging resistance mechanisms (colistin resistance *mcr-1* determinant and 16S rRNA methylase) were also characterized by PCR as previously described [23].

The formula of the Carba MTL-broth in-house medium

The composition of the medium was established on the basis of research on the various culture selective media for the isolation of *Enterobacteriaceae*. The retained formula for Carba MTL-broth in grams per litter of distilled water is as follow: Meat Peptone 10.0, Casein Peptone 5.0, Sodium Chloride 5.0, Bile Salts 9.0, Glucose 10.0, and Bromothymol Blue 0.064 (Sigma-Aldrich). The final pH was 7.8 ±0.2 at 25°C. For sterilization, we recommended boiling the media

completely for one minute without autoclaving (to avoid the Maillard reaction which modifies the colour of the media and the interpretation of the results).

Protocol experiment

The protocol consisted of inoculation of 1 mL of the Carba MTL-broth containing ertapenem, cloxacillin (250 mg/L) and vancomycin (64 mg/L) by 50 µL of each bacterial suspension (adjusted to 0.5 McFarland). Cloxacillin, which is a cephalosporinase inhibitor, was used to prevent growth of isolates expressing high levels of cephalosporinases and vancomycin was used to inhibit the growth of Gram-positive bacteria.

Table 1. Characteristic of the studied bacterial panel.

Group		Resistance profile (No. of strains)		Species (No. of strains)	β-Lactamase content (no. of strains)	MICs ETP range (mg/L)
Enterobacteriaceae		3GC[a] susceptible (1)	No resistance to □-lactams (1)	*E. coli* (1)	None	0.125
		3GC[a] resistant (46)	Extended-spectrum □-lactamases (21)	*E. coli* (16)	CTX-M-group 1[b] (11)	0.023-0.25
					CTX-M-group 9 (4)	0.004-0.032
					SHV-12 (1)	0.125
				K. pneumoniae (3)	CTX-M-group 1 (2)	0.125-0.25
					SHV-5 (1)	0.032
				P. mirabilis (1)	CTX-M-group 1 (1)	0.008
				E. aerogenes (1)	TEM-24 (1)	0.25
			Cephalosporinases/ overexpressed AmpC (25)	*E. coli* (6)	Overexpressed AmpC (4)	0.023-0.75
					CMY-4 (2)	0.125
				E. cloacae (10)	Overexpressed AmpC (9)	0.125-6
					Overexpressed AmpC , CTX-M- group 9 (1)	0.5
				K. oxytoca (3)	OXY overexpressed AmpC (3)	0.5->32
				C. freundii (3)	Overexpressed AmpC (3)	0.19-0.75
				C. koseri (1)	DHA-1 (1)	0.125
				M. morganii (1)	Overexpressed AmpC (1)	0.012
				P. mirabilis (1)	CMY-16 (1)	0.125
		Carbapenem resistant (66)	Class A carbapenemase (11)	*K. pneumoniae* (9)	KPC-2, SHV-1 (7)	8->32
					KPC-2, CTX-M-group 1, SHV-1 (1)	>32
					KPC-3, SHV-1 (1)	>32
				E. coli (1)	KPC-2, TEM-1 (1)	8
				E. cloacae (1)	IMI-1, AmpC (1)	>32
			Class B carbapenemase (11)	*E. coli* (5)	NDM-1, CTX-M-group 1 (1)	>32
					NDM-1 (1)	16
					NDM-1, DHA-1 (1)	>32
					VIM-1 (2)	6-8
				K. pneumoniae (4)	NDM-1, CTX-M-group 1, SHV-1 (4)	4->32
				E. cloacae (1)	VIM-1, Overexpressed AmpC (1)	3
				C. freundii (1)	VIM-1, Overexpressed AmpC (1)	1
			Class D carbapenemase (33)	*E. coli* (11)	OXA-48 (10)	0.38-16
					OXA-181, CTX-M-group 1 (1)	3
				K. pneumoniae (10)	OXA-48, SHV-1 (6)	4->32
					OXA-48, CTX-M-group 1, SHV-1 (4)	0.75-8
				K. oxytoca (3)	OXA-48, OXY-1 (3)	0.38-2
				E. cloacae (2)	OXA-48, CTX-M-group 1, AmpC (2)	1.5-24
				E. aerogenes (2)	OXA-48, AmpC (2)	2-6
				C. werknanii (2)	OXA-48 (2)	0.5-8
				R. ornithinolytica (1)	OXA-48 (1)	0.5
				R. planticola (1)	OXA-48 (1)	32
				P. gergoviae (1)	OXA-48 (1)	8
			Impermeability (11)	*K. pneumoniae* (9)	DHA-1, SHV-1 (8)	0.38-2
					CTX-M-group 1, SHV-1 (1)	1
				E. coli (2)	DHA-1, SHV-1 (1)	0.38
					CMY-2 (1)	0.5
Non-fermenting bacilli	**Gram-negative**	Carbapenem resistant	Class A carbapenemase (2)	*P. aeruginosa* (2)	IMP-1 (2)	NT[e]
			Class B carbapenemase (7)	*P. aeruginosa* (4)	VIM-1 (3)	NT[e]
					VIM-1, TEM-1 (1)	NT[e]
				A. baumannii (1)	NDM-1 (1)	NT[e]
				S. maltophilia (2)	L1 (2)	NT[e]
			Class D carbapenemase (11)	*A. baumannii* (11)	OXA-23 (8)	NT[e]
					OXA-24 (2)	NT[e]
					OXA-23, OXA-58 (1)	NT[e]
			Impermeability (7)	*P. aeruginosa* (6)	AmpC hyperproduction (1)	NT[e]
				A. nosocomialis (1)	-	NT[e]
Control strains						NT[e]
		MSSA[c]	ATCC29213	*S. aureus* (1)		NT[e]
		MRSA[d]	ATCC103911	*S. aureus* (1)		NT[e]
		-		*E. faecalis* (1)		NT[e]
		Van A		*E. faecium* (1)		NT[e]

[a]3GC, Third generation cephalosporin; [b] Two CTX-M-producing *E. coli* harboured the plasmid-mediated colistin resistance gene *mcr-1*; [c]MSSA, methicillin–sensitive *Staphylococcus aureus*; [d] MRSA, methicillin–resistant *Staphylococcus aureus*; [e]NT, no tested.

We added paraffin oil to create anaerobic conditions in order to inhibit carbapenemase-producing non-fermentative GNB. After incubation for 12 hours at 37°C, the tubes of Carba MTL-broth showing a colour change (green to yellow) were considered positive (Figure 1).

Validation of the best concentration of ertapenem

In order to determine the best concentration of ertapenem to use in this assay, we evaluated four different concentrations of ertapenem 0.5, 1, 1.5 and 2 mg/L. The 144 isolates of our panel were tested with this protocol. Each of the assays was repeated in triplicate.

Limits of detection of Carba MTL-broth compared with chromID OXA-48 and chromID CARBA media

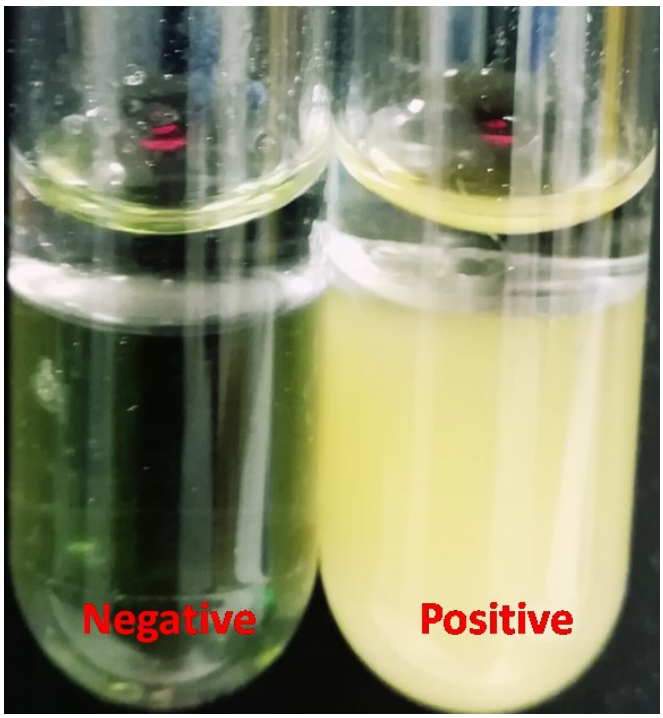
The 113 enterobacteria isolates included in this study (Table 1) were tested in order to determine the limits of detection of Carba MTL-broth compared with ChromID® CARBA and ChromID® OXA-48 (BioMérieux, Marcy l'Etoile, France). Strains were suspended in normal saline to the density of a 0.5 McFarland standard (~ 1×10^8 CFU/mL), followed by serial 7-fold dilutions. Seven inocula were tested ranging from 1×10^1 to 1×10^7 CFU/mL. Each inoculum was placed on each screening medium. Carba MTL-broth was inoculated by 50 µL of each inoculum and incubated for 12 hours at 37°C, and then enumeration of colonies was made by streaking 100 µL of the latest inoculum giving a positive result on Brain Heart Infusion Agar plates. In parallel, each inoculum was plated on each screening chromogenic medium (100 µL). Viable bacteria were counted after 24 hours of culture at 37 °C. The performance of the three selective media was compared taking into account the bacterial inoculum.

Results

Panel description concerning the ertapenem susceptibility

The results of the MICs of ertapenem for the panel of enterobacteria are shown in Table 1. Ertapenem MICs for the 55 CPE and the 11 carbapenem-resistant enterobacteria by impermeability were ranged from 0.38 to > 32 mg/L and 0.38 to 2 mg/L, respectively. This range varied between 0.004 to 0.25 mg/L for ESBLs producers, 0.125 mg/L for pAmpC producers, 0.023 mg/L to > 32 mg/L for overexpressed AmpC and 0.094 to 0.25 mg/L for *mcr-1* producers (Table 1).

Figure 1. Interpretation colors of Carba MTL-broth.



Determination of optimal ertapenem concentration for the detection of CPE

To detect the optimal ertapenem concentration, the Carba MTL-broth was supplemented with four concentrations of ertapenem varying from 0.5 to 2 mg/L. The performance of the different solutions against the 144 bacteria of the studied panel was presented in Table 2.

The ertapenem concentration of 0.5 mg/L presented the best performance for a lab test: 100 % sensitivity, 77.5 % specificity and was used for the other part of this study (Table 2).

Limits of detection of Carba MTL-broth as compared to those obtained with chromID® OXA-48 and chromID® CARBA media

The lowest limit of detection of OXA-48-like producers respectively was 1×10^1 CFU/plate and ranged from 1×10^1 to 1×10^2 CFU/plate by using the Carba MTL-broth and chromID® OXA-48 media for most of the strains, whereas it was mainly $\geq 1 \times 10^4$ CFU/plate by using the chromID® CARBA (Table 3).

The Carba MTL-broth had the highest sensitivity (100 %) for the detection of OXA-48 producers, for low and high inoculums (Table 4). The chromID® OXA-48 had also a high sensitivity for a low (10^2 CFU/plate) and a high inoculum (10^4 CFU/plate) ranging from 90.9% to 100%, respectively.

Table 2. Carba MTL-broth results using four different concentrations of ertapenem (0.5 to 2 mg/L).

			Carba MTL-broth [0.5 mg/L]			Carba MTL-broth [1 mg/L]			Carba MTL-broth [1.5 mg/L]			Carba MTL-broth [2 mg/L]		
Bacterial species	**Carbapenemase-producing enterobacteria**	**ETP MIC mg/L**	**MT 1**	**MT 2**	**MT 3**	**MT 1**	**MT 2**	**MT 3**	**MT 1**	**MT 2**	**MT 3**	**MT 1**	**MT 2**	**MT 3**
Ambler class D carbapenemases (n = 33)														
Escherichia coli[B]	OXA-48	16	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[B]	OXA-48	16	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[B]	OXA-48	8	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[B]	OXA-48	8	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[B]	OXA-48	16	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[B]	OXA-48	16	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[B]	OXA-48	16	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[N]	OXA-48	0.5	+	+	+	-	-	-	-	-	-	-	-	-
Escherichia coli[N]	OXA-48	0.38	+	+	+	-	-	-	-	-	-	-	-	-
Escherichia coli[N]	OXA-48	0.38	+	+	+	-	-	-	-	-	-	-	-	-
Escherichia coli[N]	OXA-181, CTX-M-group 1	3	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[B]	OXA-48, SHV-1	32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[B]	OXA-48, SHV-1	8	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[B]	OXA-48, SHV-1	16	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[B]	OXA-48, SHV-1	16	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	OXA-48, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	OXA-48, SHV-1	4	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	OXA-48, CTX-M-group 1, SHV-1	8	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	OXA-48, CTX-M-group 1, SHV-1	6	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	OXA-48, CTX-M-group 1, SHV-1	0.75	+	+	+	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae[N]	OXA-48, CTX-M-group 1, SHV-1	0.75	+	+	+	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca[B]	OXA-48, OXY-1	0.5	+	+	+	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca[N]	OXA-48, OXY-1	2	+	+	+	+	+	+	+	+	+	-	-	-
Klebsiella oxytoca[N]	OXA-48, OXY-1	0.38	+	+	+	-	-	-	-	-	-	-	-	-
Enterobacter cloacae[N]	OXA-48, CTX-M-group 1, AmpC	24	+	+	+	+	+	+	+	+	+	+	+	+
Enterobacter cloacae[N]	OXA-48, CTX-M-group 1, AmpC	1.5	+	+	+	+	+	+	-	-	-	-	-	-
Enterobacter aerogenes[N]	OXA-48, AmpC	6	+	+	+	+	+	+	+	+	+	+	+	+
Enterobacter aerogenes[N]	OXA-48, AmpC	2	+	+	+	+	+	+	+	+	+	-	-	-
Citrobacter werkmanii[B]	OXA-48	8	+	+	+	+	+	+	+	+	+	+	+	+
Citrobacter werkmanii[N]	OXA-48	0.5	+	+	+	-	-	-	-	-	-	-	-	-
Raoultella ornithinolytica[B]	OXA-48	0.5	+	+	+	-	-	-	-	-	-	-	-	-
Raoultela planticola[B]	OXA-48	32	+	+	+	+	+	+	+	+	+	+	+	+
Pluralibacter gergoviae[B]	OXA-48	8	+	+	+	+	+	+	+	+	+	+	+	+
Ambler class A carbapenemases (n = 11)														
Klebsiella pneumoniae[B]	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	KPC-2, SHV-1	8	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	KPC-2, CTX-M-group 1, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[B]	KPC-3, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[N]	KPC-2, TEM-1	8	+	+	+	+	+	+	+	+	+	+	+	+
Enterobacter cloacae[N]	IMI-1, AmpC	>32	+	+	+	+	+	+	+	+	+	+	+	+
Ambler class B carbapenemases (n = 11)														
Escherichia coli[N]	NDM-1, CTX-M-group 1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[N]	NDM-1	16	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[N]	NDM-1, DHA-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[B]	VIM-1	8	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli[N]	VIM-1	6	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[B]	NDM-1, CTX-M-group 1, SHV-1	4	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	NDM-1, CTX-M-group 1, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	NDM-1, CTX-M-group 1, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae[N]	NDM-1, CTX-M-group 1, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
Enterobacter cloacae[N]	VIM-1, AmpC	3	+	+	+	+	+	+	+	+	+	+	+	+
Citrobacter freundii[N]	VIM-1, AmpC	1	+	+	+	-	-	-	-	-	-	-	-	-

Table 2 (continued). Carba MTL-broth results using four different concentrations of ertapenem (0.5 to 2 mg/L).

Bacterial species	Carbapenemase-producing enterobacteria	ETP MIC mg/L	Carba MTL-broth [0.5 mg/L]			Carba MTL-broth [1 mg/L]			Carba MTL-broth [1.5 mg/L]			Carba MTL-broth [2 mg/L]		
			MT 1	MT 2	MT 3	MT 1	MT 2	MT 3	MT 1	MT 2	MT 3	MT 1	MT 2	MT 3
Non-carbapenemase producers with decreased susceptibility to carbapenems (n = 26)														
Klebsiella pneumoniae N,1	CTX-M-group 1, SHV-1	1	+	+	+	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae N,1	DHA-1, SHV-1	1.5	+	+	+	+	+	+	-	-	-	-	-	-
Klebsiella pneumoniae N,1	DHA-1, SHV-1	0.75	+	+	+	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae N,1	DHA-1, SHV-1	2	+	+	+	+	+	+	+	+	+	-	-	-
Klebsiella pneumoniae N,1	DHA-1, SHV-1	0.38	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae N,1	DHA-1, SHV-1	0.38	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae N,1	DHA-1, SHV-1	0.38	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae N,1	DHA-1, SHV-1	1	+	+	+	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae N,1	DHA-1, SHV-1	1	+	+	+	-	-	-	-	-	-	-	-	-
Escherichia coli N,1	DHA-1, SHV-1	0.38	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli N,1	CMY-2	0.5	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B,2	AmpC hyperproduction	0.75	+	+	+	-	-	-	-	-	-	-	-	-
*Escherichia coli*N,2	AmpC hyperproduction	0.5	+	+	+	-	-	-	-	-	-	-	-	-
*Enterobacter cloacae*N,2	AmpC hyperproduction	6	+	+	+	+	+	+	+	+	+	+	+	+
Enterobacter cloacae N,2	AmpC hyperproduction	3	+	+	+	+	+	+	+	+	+	+	+	+
Enterobacter cloacae N,2	AmpC hyperproduction	2	+	+	+	+	+	+	+	+	+	-	-	-
Enterobacter cloacae N,2	AmpC hyperproduction	0.5	+	+	+	-	-	-	-	-	-	-	-	-
Enterobacter cloacae N,2	AmpC hyperproduction	0.5	+	+	+	-	-	-	-	-	-	-	-	-
Enterobacter cloacae N,2	AmpC hyperproduction, CTX-M-group 9	0.5	+	+	+	-	-	-	-	-	-	-	-	-
*Enterobacter cloacae*B,2	AmpC hyperproduction	2	+	+	+	+	+	+	+	+	+	-	-	-
Enterobacter cloacae N,2	AmpC hyperproduction	0.75	+	+	+	-	-	-	-	-	-	-	-	-
Enterobacter cloacae N,2	AmpC hyperproduction	0.38	-	-	-	-	-	-	-	-	-	-	-	-
*Citrobacter freundii*B,2	AmpC hyperproduction	0.75	+	+	+	-	-	-	-	-	-	-	-	-
*Klebsiella oxytoca*N,2	OXY hyperproduction	0.5	+	+	+	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca N,2	OXY hyperproduction	>32	+	+	+	+	+	+	+	+	+	+	+	+
Klebsiella oxytoca N,2	OXY hyperproduction	12	+	+	+	+	+	+	+	+	+	+	+	+
Non-carbapenemase producers being susceptible to carbapenems (n = 32)														
*Escherichia coli*B	CTX-M-group 1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	CTX-M-group 1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	CTX-M-group 1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	CTX-M-group 1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	CTX-M-group 1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	CTX-M-group 1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	CTX-M-group 1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	CTX-M-group 1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*N	CTX-M-group 1	0.023	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	CTX-M-group 1, *mcr*-1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*N	CTX-M-group 1, *mcr*-1	0.094	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*N	CTX-M-group 9	0.004	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*N	CTX-M-group 9	0.032	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*N	CTX-M-group 9	0.006	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*N	CTX-M-group 9	0.032	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	SHV-12	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Klebsiella pneumoniae*B	CTX-M-group 1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Klebsiella pneumoniae*B	CTX-M-group 1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
*Klebsiella pneumoniae*N	SHV-5	0.032	-	-	-	-	-	-	-	-	-	-	-	-
*Proteus mirabilis*N	CTX-M-group 1	0.008	-	-	-	-	-	-	-	-	-	-	-	-
*Enterobacter aerogenes*B	TEM-24	0.25	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	AmpC hyperproduction	0.25	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*N	AmpC hyperproduction	0.023	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	CMY-4	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	CMY-4	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Enterobacter cloacae*N	AmpC hyperproduction	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Citrobacter freundii*N	AmpC hyperproduction	0.19	-	-	-	-	-	-	-	-	-	-	-	-
*Citrobacter freundii*N	AmpC hyperproduction	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Morganela morganii*N	AmpC hyperproduction	0.012	-	-	-	-	-	-	-	-	-	-	-	-
*Citrobacter koseri*B	DHA-1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Proteus mirabilis*B	CMY-16	0.125	-	-	-	-	-	-	-	-	-	-	-	-
*Escherichia coli*B	ATCC 29522	0.125	-	-	-	-	-	-	-	-	-	-	-	-

Table 2 (continued). Carba MTL-broth results using four different concentrations of ertapenem (0.5 to 2 mg/L).

			Carba MTL-broth [0.5 mg/L]			Carba MTL-broth [1 mg/L]			Carba MTL-broth [1.5 mg/L]			Carba MTL-broth [2 mg/L]		
Bacterial species	**Carbapenemase-producing enterobacteria**	**ETP MIC mg/L**	**MT 1**	**MT 2**	**MT 3**	**MT 1**	**MT 2**	**MT 3**	**MT 1**	**MT 2**	**MT 3**	**MT 1**	**MT 2**	**MT 3**
Non-fermenting Gram-negative bacilli (n = 27)														
Pseudomonas aeruginosa[N]	IMP-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	IMP-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	VIM-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	VIM-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	VIM-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	VIM-1, TEM-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	Overproduction AmpC	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa[N]	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	NDM-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-23, OXA-58	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-24	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii[N]	OXA-24	NT	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter nosocomialis[N]	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
Stenotrophomonas maltophilia[N]	L1	NT	-	-	-	-	-	-	-	-	-	-	-	-
Stenotrophomonas maltophilia[N]	L1	NT	-	-	-	-	-	-	-	-	-	-	-	-
Gram-positive bacteria (n = 4)														
MSSA[B]	ATCC 29213	NT	-	-	-	-	-	-	-	-	-	-	-	-
MRSA[B]	ATCC 103911	NT	-	-	-	-	-	-	-	-	-	-	-	-
Enterococcus faecalis[B]	Vancomycin sensitive	NT	-	-	-	-	-	-	-	-	-	-	-	-
Enterococcus faecalis[B]	Vancomycin resistant	NT	-	-	-	-	-	-	-	-	-	-	-	-
SN (%)				100			81.8			80			76.3	
SP (%)				77.5			91			92.1			95.5	
PPV (%)				73.3			84.9			86.2			91.3	
NPV (%)				100			89			88.1			86.7	

SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value; MRSA, *Staphylococcus aureus* methicillin resistant; MSSA, *Staphylococcus aureus* methicillin sensitive

Table 3. Limits of detection of Carba MTL-broth as compared to those obtained with chromID OXA-48 and chromID CARBA media.

Bacterial species	**Carbapenemase-producing enterobacteria**	**ETP MIC mg/L**	**Lowest detection limit (CFU/plate)**		
			Carba MTL-broth	**chromID OXA-48**	**chromID CARBA**
Ambler class D carbapenemases (n = 33)					
Escherichia coli[B]	OXA-48	16	1×10^1	1×10^1	1×10^5
Escherichia coli[B]	OXA-48	16	1×10^1	1×10^1	1×10^5
Escherichia coli[B]	OXA-48	8	1×10^1	1×10^1	1×10^5
Escherichia coli[B]	OXA-48	8	1×10^1	1×10^1	1×10^5
Escherichia coli[B]	OXA-48	16	1×10^1	1×10^1	1×10^5
Escherichia coli[B]	OXA-48	16	1×10^1	1×10^1	1×10^5
Escherichia coli[B]	OXA-48	16	1×10^1	1×10^1	1×10^5
Escherichia coli[N]	OXA-48	0.5	1×10^1	1×10^2	$> 1 \times 10^7$
Escherichia coli[N]	OXA-48	0.38	1×10^1	1×10^3	$> 1 \times 10^7$
Escherichia coli[N]	OXA-48	0.38	1×10^1	1×10^4	$> 1 \times 10^7$
Escherichia coli[N]	OXA-181, CTX-M-group 1	3	1×10^1	1×10^1	1×10^4
Klebsiella pneumoniae[B]	OXA-48, SHV-1	32	1×10^1	1×10^1	1×10^1
Klebsiella pneumoniae[B]	OXA-48, SHV-1	8	1×10^1	1×10^1	1×10^5
Klebsiella pneumoniae[B]	OXA-48, SHV-1	16	1×10^1	1×10^1	1×10^4
Klebsiella pneumoniae[B]	OXA-48, SHV-1	16	1×10^1	1×10^1	1×10^4
Klebsiella pneumoniae[N]	OXA-48, SHV-1	>32	1×10^1	1×10^1	1×10^1
Klebsiella pneumoniae[N]	OXA-48, SHV-1	4	1×10^1	1×10^1	1×10^5
Klebsiella pneumoniae[N]	OXA-48, CTX-M-group 1, SHV-1	8	1×10^1	1×10^1	1×10^5
Klebsiella pneumoniae[N]	OXA-48, CTX-M-group 1, SHV-1	6	1×10^1	1×10^1	1×10^5
Klebsiella pneumoniae[N]	OXA-48, CTX-M-group 1, SHV-1	0.75	1×10^1	1×10^2	1×10^6
Klebsiella pneumoniae[N]	OXA-48, CTX-M-group 1, SHV-1	0.75	1×10^1	1×10^2	1×10^7
Klebsiella oxytoca[B]	OXA-48, OXY-1	0.5	1×10^1	1×10^2	1×10^7
Klebsiella oxytoca[N]	OXA-48, OXY-1	2	1×10^1	1×10^2	1×10^5
Klebsiella oxytoca[N]	OXA-48, OXY-1	0.38	1×10^1	1×10^4	1×10^7
Enterobacter cloacae[N]	OXA-48, CTX-M-group 1, AmpC	24	1×10^1	1×10^1	1×10^2
Enterobacter cloacae[N]	OXA-48, CTX-M-group 1, AmpC	1.5	1×10^1	1×10^2	1×10^5
Enterobacter aerogenes[N]	OXA-48, AmpC	6	1×10^1	1×10^1	1×10^4
Enterobacter aerogenes[N]	OXA-48, AmpC	2	1×10^1	1×10^2	1×10^5
Citrobacter werkmanii[B]	OXA-48	8	1×10^1	1×10^1	1×10^5
Citrobacter werkmanii[N]	OXA-48	0.5	1×10^1	1×10^2	$> 1 \times 10^7$
Raoultella ornithinolytica[B]	OXA-48	0.5	1×10^1	1×10^2	$> 1 \times 10^7$
Raoultella planticola[B]	OXA-48	32	1×10^1	1×10^1	1×10^3
Pluralibacter gergoviae[B]	OXA-48	8	1×10^1	1×10^1	1×10^5
Ambler class A carbapenemases (n = 11)					
Klebsiella pneumoniae[B]	KPC-2, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[N]	KPC-2, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[N]	KPC-2, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[N]	KPC-2, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[N]	KPC-2, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[N]	KPC-2, SHV-1	8	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[N]	KPC-2, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[N]	KPC-2, CTX-M-group 1, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[B]	KPC-3, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Escherichia coli[N]	KPC-2, TEM-1	8	1×10^1	$>1 \times 10^7$	1×10^1
Enterobacter cloacae[N]	IMI-1, AmpC	>32	1×10^1	$>1 \times 10^7$	1×10^1
Ambler class B carbapenemases (n = 11)					
Escherichia coli[N]	NDM-1, CTX-M-group 1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Escherichia coli[N]	NDM-1	16	1×10^1	$>1 \times 10^7$	1×10^1
Escherichia coli[N]	NDM-1, DHA-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Escherichia coli[B]	VIM-1	8	1×10^1	$>1 \times 10^7$	1×10^1
Escherichia coli[N]	VIM-1	6	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[B]	NDM-1, CTX-M-group 1, SHV-1	4	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[N]	NDM-1, CTX-M-group 1, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[N]	NDM-1, CTX-M-group 1, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Klebsiella pneumoniae[N]	NDM-1, CTX-M-group 1, SHV-1	>32	1×10^1	$>1 \times 10^7$	1×10^1
Enterobacter cloacae[N]	VIM-1, AmpC	3	1×10^1	$>1 \times 10^7$	1×10^4
Citrobacter freundii[N]	VIM-1, AmpC	1	1×10^1	$>1 \times 10^7$	1×10^4

Table 3 (continued). Limits of detection of Carba MTL-broth as compared to those obtained with chromID OXA-48 and chromID CARBA media.

Bacterial species	Carbapenemase-producing enterobacteria	ETP MIC mg/L	Lowest detection limit (CFU/plate)		
			Carba MTL-broth	chromID OXA-48	chromID CARBA
Non-carbapenemase producers with decreased susceptibility to carbapenems (n = 26)					
Klebsiella pneumoniae [N,1]	CTX-M-group 1, SHV-1	1	1×10^1	$> 1 \times 10^7$	1×10^7
Klebsiella pneumoniae [N,1]	DHA-1, SHV-1	1.5	1×10^1	$> 1 \times 10^7$	1×10^6
Klebsiella pneumoniae [N,1]	DHA-1, SHV-1	0.75	1×10^1	$> 1 \times 10^7$	$> 1 \times 10^7$
Klebsiella pneumoniae [N,1]	DHA-1, SHV-1	2	1×10^1	$> 1 \times 10^7$	1×10^6
Klebsiella pneumoniae [N,1]	DHA-1, SHV-1	0.38	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Klebsiella pneumoniae [N,1]	DHA-1, SHV-1	0.38	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Klebsiella pneumoniae [N,1]	DHA-1, SHV-1	0.38	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Klebsiella pneumoniae [N,1]	DHA-1, SHV-1	1	1×10^1	$> 1 \times 10^7$	1×10^6
Klebsiella pneumoniae [N,1]	DHA-1, SHV-1	1	1×10^1	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli [N,1]	DHA-1, SHV-1	0.38	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli [N,1]	CMY-2	0.5	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B,2]	AmpC hyperproduction	0.75	1×10^1	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[N,2]	AmpC hyperproduction	0.5	1×10^1	$> 1 \times 10^7$	$> 1 \times 10^7$
Enterobacter cloacae[N,2]	AmpC hyperproduction	6	1×10^1	$> 1 \times 10^7$	1×10^3
Enterobacter cloacae [N,2]	AmpC hyperproduction	3	1×10^1	$> 1 \times 10^7$	1×10^5
Enterobacter cloacae [N,2]	AmpC hyperproduction	2	1×10^1	$> 1 \times 10^7$	1×10^6
Enterobacter cloacae [N,2]	AmpC hyperproduction	0.5	1×10^1	$> 1 \times 10^7$	$> 1 \times 10^7$
Enterobacter cloacae [N,2]	AmpC hyperproduction	0.5	1×10^1	$> 1 \times 10^7$	$> 1 \times 10^7$
Enterobacter cloacae [N,2]	AmpC hyperproduction, CTX-M-group 9	0.5	1×10^1	$> 1 \times 10^7$	$> 1 \times 10^7$
Enterobacter cloacae[B,2]	AmpC hyperproduction	2	1×10^1	$> 1 \times 10^7$	1×10^6
Enterobacter cloacae [N,2]	AmpC hyperproduction	0.75	1×10^1	$> 1 \times 10^7$	1×10^7
Enterobacter cloacae [N,2]	AmpC hyperproduction	0.38	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Citrobacter freundii[B,2]	AmpC hyperproduction	0.75	1×10^1	$> 1 \times 10^7$	$> 1 \times 10^7$
Klebsiella oxytoca[N,2]	OXY hyperproduction	0.5	1×10^1	$> 1 \times 10^7$	$> 1 \times 10^7$
Klebsiella oxytoca [N,2]	OXY hyperproduction	>32	1×10^1	$> 1 \times 10^7$	1×10^1
Klebsiella oxytoca [N,2]	OXY hyperproduction	12	1×10^1	$> 1 \times 10^7$	1×10^1
Non-carbapenemase producers being susceptible to carbapenems (n = 32)					
Escherichia coli[B]	CTX-M-group 1	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	CTX-M-group 1	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	CTX-M-group 1	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	CTX-M-group 1	0.25	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	CTX-M-group 1	0.25	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	CTX-M-group 1	0.25	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	CTX-M-group 1	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	CTX-M-group 1	0.25	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[N]	CTX-M-group 1	0.023	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	CTX-M-group 1, *mcr*-1	0.25	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[N]	CTX-M-group 1, *mcr*-1	0.094	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[N]	CTX-M-group 9	0.004	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[N]	CTX-M-group 9	0.032	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[N]	CTX-M-group 9	0.006	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[N]	CTX-M-group 9	0.032	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	SHV-12	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Klebsiella pneumoniae[B]	CTX-M-group 1	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Klebsiella pneumoniae[B]	CTX-M-group 1	0.25	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Klebsiella pneumoniae[N]	SHV-5	0.032	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Proteus mirabilis[N]	CTX-M-group 1	0.008	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Enterobacter aerogenes[B]	TEM-24	0.25	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	AmpC hyperproduction	0.25	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[N]	AmpC hyperproduction	0.023	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	CMY-4	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	CMY-4	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Enterobacter cloacae[N]	AmpC hyperproduction	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Citrobacter freundii[N]	AmpC hyperproduction	0.19	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Citrobacter freundii[N]	AmpC hyperproduction	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Morganela morganii[N]	AmpC hyperproduction	0.012	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Citrobacter koseri[B]	DHA-1	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Proteus mirabilis[B]	CMY-16	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$
Escherichia coli[B]	ATCC 29522	0.125	$> 1 \times 10^7$	$> 1 \times 10^7$	$> 1 \times 10^7$

ETP, ertapenem; CFU counts are considered as negative results with a cut off values set at 1×10^3 CFU/plate for calculation of sensitivity and with a cut off value at $> 1 \times 10^7$ CFU/plate for calculation of specificity; [1]Reduced susceptibility to ertapenem due to porin deficiency; [2]Reduced susceptibility to ertapenem due to overexpressed AmpC ; [B] Strains from Microbial Ecology Laboratory, Béjaia University, Béjaia, Algeria; [N] Strains from regional multidrug resistant GNB Reference Lab (the CARBA-LR group) in the French

Table 4. Sensitivity of Carba MTL-broth, chromID OXA-48, chromID CARBA media, and combination of both chromID OXA-48 and chromID CARBA to low and high inoculums for detecting Ambler class D carbapenemase producers (n = 33).

Inoculum (CFU/plate)	Sensitivity (%)			
	Carba MTL-broth	chromID OXA-48	chromID CARBA	chromID OXA-48 and chromID CARBA
10^1	100	63.7	6	63.7
10^2	100	90.9	9	90.9
10^3	100	93.9	12.1	93.9
10^4	100	100	21.1	100

When using a very low inoculum (10^2 CFU/mL [10^1 CFU/plate]), Carba MTL-broth had a higher sensitivity than the chromID® OXA-48 being 100% versus 63.7%. The chromID® CARBA had a low sensitivity, ranging from 9% to 21.2% for the detection of OXA-48-like producers whatever the inoculum was, for a low (10^2 CFU/plate) and a high inoculum (10^4 CFU/plate) respectively (Table 4). Specificity of chromID® OXA-48 was the highest with 100%, as compared to 65.5% and 84.4% for the Carba MTL-broth and chromID® CARBA media, respectively (Table 5).

Discussion

In developing countries, detection of CPE isolates is a major challenge in the routine microbiology laboratories allowing clinicians to appropriate antibiotic administration and minimizing treatment failure. It is important to recognize that no currently described phenotypic methods are able to detect all carbapenemase types, and several are associated with very poor positive predictive values for carbapenemases detection [24]. In addition, molecular-based methods for characterization of CPE are restrictive to use due to its high cost, the requirement of skilled and experienced technicians and the inability to detect new carbapenemase-encoding genes [15]. The situation is worse in developing countries due to unavailability and/or high costs of most of these methods. Thereby, we developed a new medium which should be able to detect CPE isolates with low-level resistance to carbapenems and be as selective as possible, which meets these criteria: method incorporating available supplies, simple to prepare and use, inexpensive and having a good positive predictive values for carbapenemases detection with a special focus on OXA-48 producers. The Carba MTL-broth developed in this study meets these criteria and constitutes an alternative method which can be implemented in different laboratories to detect CPE isolates.

Comparative evaluation of the in-house Carba MTL-broth developed in our study with chromID® OXA-48 and chromID® CARBA showed that Carba MTL-broth and chromID® CARBA were effective for detecting all carbapenemase groups such as VIM, KPC and OXA-48, as compared to chromID® OXA-48 which was not intended to detect other carbapenemases than OXA-48-like producers. chromID® OXA-48 and Carba MTL-broth had the highest sensitivity for detecting OXA-48 producers (93.9 % and 100 %)

Table 5. Sensitivity and specificity of Carba MTL-broth, chromID OXA-48, chromID CARBA media, and combination of both chromID OXA-48 and chromID CARBA.

	Carba MTL-broth	chromID OXA-48	chromID CARBA	chromID OXA-48 and chromID CARBA
Sensibility (%)	100	56.3	43.6	92.7
Sensibility class D carbapenemase	100	93.9	21.2	93.9
Sensibility other classes of carbapenemases (A and B)	100	0	90.9	90.9
Specificity (%)	65.5	100	84.4	84.4
Specificity reduced susceptibility (%)	23	100	40	40
Specificity susceptible	100	100	100	100
PPV (%)	73.3	100	88.8	100
NPV (%)	100	70.7	63.9	93.5

SN = sensitivity determined with cut off values set at 1×10^3 CFU/plate for each Ambler class of carbapenemase: class A is of KPC and IMI-types; class B, VIM and NDM-types; class D, OXA-48-type. SP = specificity determined with cut off values set at $> 1 \times 10^7$ CFU/plate for non-carbapenemase producers susceptible or with reduced susceptibility to carbapenems.

comparatively to chromID® CARBA (21.2 %). The chromID® OXA-48 had the highest specificity, with 100%, as compared to 65.5% and 84.4% for the Carba MTL-broth and chromID® CARBA. These results are similar to those reported by Girlich *et al*. [25]. Even if the specificity of Carba MTL-broth was low, its Negative Predictive Value was high (%) allowing to the test not to miss the CPE detection.

The Carba MTL-broth and the combination of chromID® CARBA/ chromID® OXA-48 media offer the highest sensitivities for detecting any type of carbapenemases. It is important to note that chromogenic media are not always available in developing countries such as Algeria yet. Furthermore, a combination of two chromogenic media for improving the sensitivity of the test increases the cost: 1.45 €/plate of chromID® CARBA (bioMérieux) and 1.72 €/plate of chromID OXA-48 (bioMérieux) vs 0.5 € for Carba MTL-broth.

Conclusion

We propose here an in-house Carba MTL-broth screening medium that may detect not only KPC and MBL producers but also OXA-48 producers, which have variable susceptibility to carbapenems. The Carba MTL-broth represents a significant improvement compared to the available screening media to detect CPE isolates into a single protocol, able to inform both treatment and infection prevention and control strategies in developing countries.

Acknowledgements

JPL, AS and AP belonged to the FHU InCh (Federation Hospitalo Universitaire Infections Chroniques, Aviesan) and the Thematique Phare (CHU Nîmes). AP, CDR, AS and JPL are supported by the University Hospital Nîmes (Thématique Phare 2018). AT and JPL obtained the PHC Tassili grant (grant number 17MDU980). AM received a grant from PROFAS B+ 2018 (73-PROFAS B+).

References

1. Logan LK, Weinstein RA (2017) The epidemiology of carbapenem-resistant *Enterobacteriaceae*: The impact ad evolution of a global menace. J Infect Dis 215 Suppl 1: 28-36.
2. Nordmann P, Dortet L, Poirel L (2012) Carbapenem resistance in *Enterobacteriaceae*: here is the storm! Trends Mol Med 18: 263–272.
3. Nordmann P, Poirel L, Walsh TR, Livermore DM (2001) The emerging NDM carbapenemases. Trends Microbiol 19: 588-595.
4. Nordmann P, Poirel L (2013) Strategies for identification of carbapenemase-producing *Entrobacteriaceae*. J Antimicrob Chemother 68: 487-489.
5. Van Duin D, Doi Y (2017) The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. Virulence 8: 460-469.
6. Girlich D, Anglade C, Zambardi G, Nordmann P (2013) Comparative evaluation of a novel chromogenic medium (chromID OXA-48) for detection of OXA-48 producing *Enterobacteriaceae*. Diagn Microbiol Infect Dis 77: 296–300.
7. Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H, Greko C, So AD, Bigdeli M, Tomson G, Woodhouse W, Ombaka E, Peralta AQ, Qamar FN, Mir F, Kariuki S, Bhutta ZA, Coates A, Bergstrom R, Wright GD, Brown ED, Cars O (2013) Antibiotic resistance-the need for global solutions. Lancet Infect Dis 13: 1057–1098.
8. Mairi A, Pantel A, Sotto A, Lavigne JP, Touati A (2018) OXA-48-like carbapenemases producing *Enterobacteriaceae* in different niches. Eur J Clin Microbiol Infect Dis 37: 587–604.
9. Tamma PD, Simner PJ (2018) Phenotypic detection of carbapenemase-producing organisms from clinical isolates. J Clin Microbiol 56: pii: e01140-1118.
10. Banerhee R, Humphries R (2017) Clinical and laboratory considerations for the rapid detection of carbapenem-resistant *Enterobacteriaceae*. Virulence 8: 427-439.
11. Birgy A, Bidet P, Genel N, Doit C, Decré D, Arlet G, Bingen E (2012) Phenotypic screening of carbapenemases and associated b-lactamases in carbapenem-resistant *Enterobacteriaceae*. J Clin Microbiol 50: 1295-1302.
12. Doyle D, Peirano G, LAscols C, Lloyd T, Church DL, Pitout JDD (2012) Laboratory detection of *Enterobacteriaceae* that produce carbapenemases. J Clin Microbiol 50: 3877-3880.
13. Nordmann P, Poirel L, Dortet L (2012) Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 18: 1503-1507.
14. Pantel A, Souzy D, Sotto A, Lavigne JP (2015) Evaluation of two phenotypic screening tests for carbapenemase-producing *Enterobacteriaceae*. J Clin Microbiol 53: 3359-3362.
15. Teethaisong Y, Eumkeb G, Nakouti I, Evans K, Hobbs G (2016) A combined disc method with resazurin agar plate assay for early phenotypic screening of KPC, MBL and OXA-48 carbapenemases among *Enterobacteriaceae*. J Appl Microbiol 121: 408–414.
16. Zarakolu P, Day KM, Sidjabat HE, Kamolvit W, Lanyon CV, Cummings SP, Paterson DL, Akova M, Perry JD (2015) Evaluation of a new chromogenic medium, chromID OXA-48, for recovery of carbapenemase-producing *Enterobacteriaceae* from patients at a university hospital in Turkey. Eur J Clin Microbiol Infect Dis 34: 519–525.
17. Agabou A, Pantel A, Ouchenane Z, Lezzar N, Khemissi S, Satta D, Sotto A, Lavigne JP. (2014) First description of OXA-48-producing *Escherichia coli* and the pandemic clone ST131

from patients hospitalised at a military hospital in Algeria. Eur J Clin Microbiol Infect Dis 33: 1641-1646.
18. Mairi A, Touati A, Ait Bessai S, Boutabtoub Y, Khelifi F, Sotto A, Lavigne JP, Pantel A (2019) Carbapenemase-producing *Enterobacteriaceae* among pregnant women and newborns in Algeria: Prevalence, molecular characterization, maternal-neonatal transmission, and risk factors for carriage. Am J Infect Control 47: 105-108.
19. Pantel A, Boutet-Dubois A, Jean-Pierre H, Marchandin H, Sotto A, Lavigne JP, and CARB-LR group (2014) French Regional surveillance program of carbapenemase-producing Gram-negative bacilli: Result of two-years period. Eur J Clin Microbiol Infect Dis 33: 2285-2292.
20. Robert J, Pantel A, Merens A, Meiller E, Lavigne JP, Nicolas-Chanoine MH, ONERBA's carbapenem resistance study group (2017) Development of an algorithm for phenotypic screening of carbapenemase-producing *Enterobacteriaceae* in the routine laboratory. BMC Infect Dis 17: 78.
21. Aguirre-Quiñonero A, Martinez-Martinez L (2017) Non-molecular detection of carbapenemases in Enterobacteriaceae clinical isolates. J Infect Chemother 23: 1-11.
22. Doi Y, Paterson DL (2015) Carbapenemase-producing *Enterobacteriaceae*. Semin Respir Crit Care Med 36: 74-84.
23. Bontron S, Poirel L, Nordmann P (2016) Real-time PCR for detection of plasmid-mediated polymyxin resistance (mcr-1) from cultured bacteria and stools. J Antimicrob Chemother 71: 2318–2320.
24. Miller S, Humphries RM (2016) Clinical laboratory detection of carbapenem-resistant and carbapenemase-producing *Enterobacteriaceae*. Expert Rev Anti Infect Ther 14: 705–717.
25. Girlich D, Poirel L, Nordmann P (2013) Comparison of the SUPERCARBA, CHROMagar KPC, and Brilliance CRE screening media for detection of *Enterobacteriaceae* with reduced susceptibility to carbapenems. Diagn Microbiol Infect Dis 75: 214–217.

Corresponding author

Professor Jean-Philippe Lavigne, MD. PhD
Service de Microbiologie, CHU Caremeau, Place du Professeur Robert Debre,
30029 Nîmes cedex 09
France
Tel: +33-466683202
Fax: +33-466684254
Email: jean.philippe.lavigne@chu-nimes.fr

Conflict of interests: No conflict of interests is declared.