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Original Article

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

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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.

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

^a3GC, Third generation cephalosporin; ^b Two CTX-M-producing *E. coli* harboured the plasmid-mediated colistin resistance gene *mcr-1*; ^cMSSA, methicillin-sensitive *Staphylococcus aureus*; ^d MRSA, methicillin-resistant *Staphylococcus aureus*; ^eNT, 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

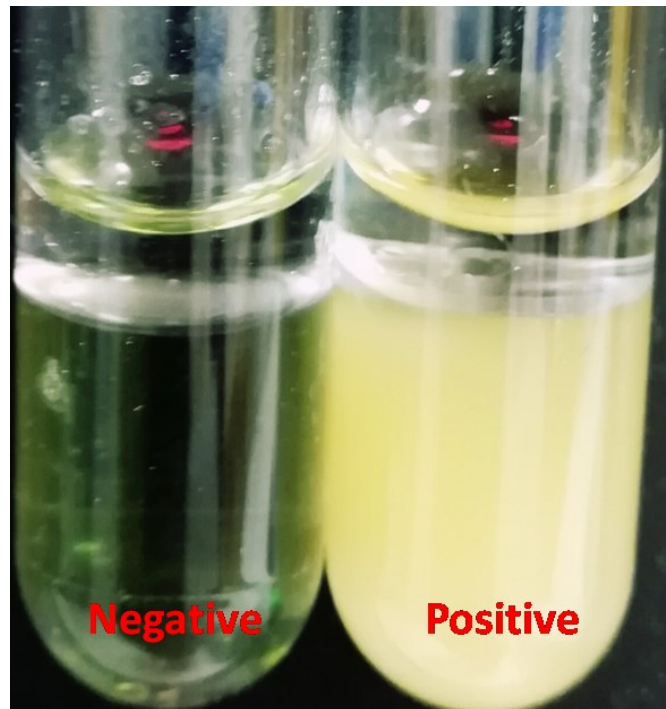
The 113 enterobacteria isolates included in this study (Table 1) were tested in order to determine the limits of detection of Carba MTL-broth compared with ChromID® CARBA and ChromID® OXA-48 (BioMérieux, Marcy l'Etoile, France). Strains were suspended in normal saline to the density of a 0.5 McFarland standard ($\sim 1 \times 10^8$ 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 μ L of each inoculum and incubated for 12 hours at 37°C, and then enumeration of colonies was made by streaking 100 μ L 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 μ L). 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).

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
Ambler class D carbapenemases (n = 33)														
<i>Escherichia coli</i> ^B	OXA-48	16	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^B	OXA-48	16	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^B	OXA-48	8	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^B	OXA-48	8	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^B	OXA-48	16	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^B	OXA-48	16	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^B	OXA-48	16	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^N	OXA-48	0.5	+	+	+	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^N	OXA-48	0.38	+	+	+	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^N	OXA-48	0.38	+	+	+	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^N	OXA-181, CTX-M-group 1	3	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^B	OXA-48, SHV-1	32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^B	OXA-48, SHV-1	8	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^B	OXA-48, SHV-1	16	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^B	OXA-48, SHV-1	16	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	OXA-48, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	OXA-48, SHV-1	4	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	OXA-48, CTX-M-group 1, SHV-1	8	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	OXA-48, CTX-M-group 1, SHV-1	6	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	OXA-48, CTX-M-group 1, SHV-1	0.75	+	+	+	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^N	OXA-48, CTX-M-group 1, SHV-1	0.75	+	+	+	-	-	-	-	-	-	-	-	-
<i>Klebsiella oxytoca</i> ^B	OXA-48, OXY-1	0.5	+	+	+	-	-	-	-	-	-	-	-	-
<i>Klebsiella oxytoca</i> ^N	OXA-48, OXY-1	2	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella oxytoca</i> ^N	OXA-48, OXY-1	0.38	+	+	+	-	-	-	-	-	-	-	-	-
<i>Enterobacter cloacae</i> ^N	OXA-48, CTX-M-group 1, AmpC	24	+	+	+	+	+	+	+	+	+	+	+	+
<i>Enterobacter cloacae</i> ^N	OXA-48, CTX-M-group 1, AmpC	1.5	+	+	+	+	+	+	-	-	-	-	-	-
<i>Enterobacter aerogenes</i> ^N	OXA-48, AmpC	6	+	+	+	+	+	+	+	+	+	+	+	+
<i>Enterobacter aerogenes</i> ^N	OXA-48, AmpC	2	+	+	+	+	+	+	+	+	+	+	+	+
<i>Citrobacter werkmanii</i> ^B	OXA-48	8	+	+	+	+	+	+	+	+	+	+	+	+
<i>Citrobacter werkmanii</i> ^N	OXA-48	0.5	+	+	+	-	-	-	-	-	-	-	-	-
<i>Raoultella ornithinolytica</i> ^B	OXA-48	0.5	+	+	+	-	-	-	-	-	-	-	-	-
<i>Raoultella planticola</i> ^B	OXA-48	32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Pluralibacter gergoviae</i> ^B	OXA-48	8	+	+	+	+	+	+	+	+	+	+	+	+
Ambler class A carbapenemases (n = 11)														
<i>Klebsiella pneumoniae</i> ^B	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	8	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	KPC-2, CTX-M-group 1, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^B	KPC-3, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^N	KPC-2, TEM-1	8	+	+	+	+	+	+	+	+	+	+	+	+
<i>Enterobacter cloacae</i> ^N	IMI-1, AmpC	>32	+	+	+	+	+	+	+	+	+	+	+	+
Ambler class B carbapenemases (n = 11)														
<i>Escherichia coli</i> ^N	NDM-1, CTX-M-group 1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^N	NDM-1	16	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^N	NDM-1, DHA-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^B	VIM-1	8	+	+	+	+	+	+	+	+	+	+	+	+
<i>Escherichia coli</i> ^N	VIM-1	6	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^B	NDM-1, CTX-M-group 1, SHV-1	4	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	NDM-1, CTX-M-group 1, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	NDM-1, CTX-M-group 1, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i> ^N	NDM-1, CTX-M-group 1, SHV-1	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Enterobacter cloacae</i> ^N	VIM-1, AmpC	3	+	+	+	+	+	+	+	+	+	+	+	+
<i>Citrobacter freundii</i> ^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)														
<i>Klebsiella pneumoniae</i> ^{N,1}	CTX-M-group 1, SHV-1	1	+	+	+	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	1.5	+	+	+	+	+	+	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	0.75	+	+	+	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	2	+	+	+	+	+	+	+	+	+	-	-	-
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	0.38	-	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	0.38	-	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	0.38	-	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	1	+	+	+	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	1	+	+	+	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^{N,1}	DHA-1, SHV-1	0.38	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^{N,1}	CMY-2	0.5	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^{B,2}	AmpC hyperproduction	0.75	+	+	+	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^{N,2}	AmpC hyperproduction	0.5	+	+	+	-	-	-	-	-	-	-	-	-
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	6	+	+	+	+	+	+	+	+	+	+	+	+
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	3	+	+	+	+	+	+	+	+	+	+	+	+
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	2	+	+	+	+	+	+	+	+	+	-	-	-
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	0.5	+	+	+	-	-	-	-	-	-	-	-	-
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	0.5	+	+	+	-	-	-	-	-	-	-	-	-
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction, CTX-M-group 9	0.5	+	+	+	-	-	-	-	-	-	-	-	-
<i>Enterobacter cloacae</i> ^{B,2}	AmpC hyperproduction	2	+	+	+	+	+	+	+	+	+	-	-	-
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	0.75	+	+	+	-	-	-	-	-	-	-	-	-
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	0.38	-	-	-	-	-	-	-	-	-	-	-	-
<i>Citrobacter freundii</i> ^{B,2}	AmpC hyperproduction	0.75	+	+	+	-	-	-	-	-	-	-	-	-
<i>Klebsiella oxytoca</i> ^{N,2}	OXY hyperproduction	0.5	+	+	+	-	-	-	-	-	-	-	-	-
<i>Klebsiella oxytoca</i> ^{N,2}	OXY hyperproduction	>32	+	+	+	+	+	+	+	+	+	+	+	+
<i>Klebsiella oxytoca</i> ^{N,2}	OXY hyperproduction	12	+	+	+	+	+	+	+	+	+	+	+	+
Non-carbapenemase producers being susceptible to carbapenems (n = 32)														
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^N	CTX-M-group 1	0.023	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	CTX-M-group 1, <i>mcr-1</i>	0.25	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^N	CTX-M-group 1, <i>mcr-1</i>	0.094	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^N	CTX-M-group 9	0.004	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^N	CTX-M-group 9	0.032	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^N	CTX-M-group 9	0.006	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^N	CTX-M-group 9	0.032	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	SHV-12	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^B	CTX-M-group 1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^B	CTX-M-group 1	0.25	-	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ^N	SHV-5	0.032	-	-	-	-	-	-	-	-	-	-	-	-
<i>Proteus mirabilis</i> ^N	CTX-M-group 1	0.008	-	-	-	-	-	-	-	-	-	-	-	-
<i>Enterobacter aerogenes</i> ^B	TEM-24	0.25	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	AmpC hyperproduction	0.25	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^N	AmpC hyperproduction	0.023	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	CMY-4	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	CMY-4	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Enterobacter cloacae</i> ^N	AmpC hyperproduction	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Citrobacter freundii</i> ^N	AmpC hyperproduction	0.19	-	-	-	-	-	-	-	-	-	-	-	-
<i>Citrobacter freundii</i> ^N	AmpC hyperproduction	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Morganella morganii</i> ^N	AmpC hyperproduction	0.012	-	-	-	-	-	-	-	-	-	-	-	-
<i>Citrobacter koseri</i> ^B	DHA-1	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Proteus mirabilis</i> ^B	CMY-16	0.125	-	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i> ^B	ATCC 29522	0.125	-	-	-	-	-	-	-	-	-	-	-	-

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-fermenting Gram-negative bacilli (n = 27)														
<i>Pseudomonas aeruginosa</i> ^N	IMP-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	IMP-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	VIM-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	VIM-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	VIM-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	VIM-1, TEM-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	Overproduction AmpC	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ^N	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	NDM-1	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-23, OXA-58	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-24	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-24	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter baumannii</i> ^N	OXA-24	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter nosocomialis</i> ^N	Impermeability decrease	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Stenotrophomonas maltophilia</i> ^N	L1	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Stenotrophomonas maltophilia</i> ^N	L1	NT	-	-	-	-	-	-	-	-	-	-	-	-
Gram-positive bacteria (n = 4)														
MSSA ^B	ATCC 29213	NT	-	-	-	-	-	-	-	-	-	-	-	-
MRSA ^B	ATCC 103911	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Enterococcus faecalis</i> ^B	Vancomycin sensitive	NT	-	-	-	-	-	-	-	-	-	-	-	-
<i>Enterococcus faecalis</i> ^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)					
<i>Escherichia coli</i> ^B	OXA-48	16	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Escherichia coli</i> ^B	OXA-48	16	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Escherichia coli</i> ^B	OXA-48	8	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Escherichia coli</i> ^B	OXA-48	8	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Escherichia coli</i> ^B	OXA-48	16	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Escherichia coli</i> ^B	OXA-48	16	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Escherichia coli</i> ^B	OXA-48	16	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Escherichia coli</i> ^N	OXA-48	0.5	1 × 10 ¹	1 × 10 ²	> 1 × 10 ⁷
<i>Escherichia coli</i> ^N	OXA-48	0.38	1 × 10 ¹	1 × 10 ³	> 1 × 10 ⁷
<i>Escherichia coli</i> ^N	OXA-48	0.38	1 × 10 ¹	1 × 10 ⁴	> 1 × 10 ⁷
<i>Escherichia coli</i> ^N	OXA-181, CTX-M-group 1	3	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁴
<i>Klebsiella pneumoniae</i> ^B	OXA-48, SHV-1	32	1 × 10 ¹	1 × 10 ¹	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^B	OXA-48, SHV-1	8	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Klebsiella pneumoniae</i> ^B	OXA-48, SHV-1	16	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁴
<i>Klebsiella pneumoniae</i> ^B	OXA-48, SHV-1	16	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁴
<i>Klebsiella pneumoniae</i> ^N	OXA-48, SHV-1	>32	1 × 10 ¹	1 × 10 ¹	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	OXA-48, SHV-1	4	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Klebsiella pneumoniae</i> ^N	OXA-48, CTX-M-group 1, SHV-1	8	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Klebsiella pneumoniae</i> ^N	OXA-48, CTX-M-group 1, SHV-1	6	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Klebsiella pneumoniae</i> ^N	OXA-48, CTX-M-group 1, SHV-1	0.75	1 × 10 ¹	1 × 10 ²	1 × 10 ⁶
<i>Klebsiella pneumoniae</i> ^N	OXA-48, CTX-M-group 1, SHV-1	0.75	1 × 10 ¹	1 × 10 ²	1 × 10 ⁷
<i>Klebsiella oxytoca</i> ^B	OXA-48, OXY-1	0.5	1 × 10 ¹	1 × 10 ²	1 × 10 ⁷
<i>Klebsiella oxytoca</i> ^N	OXA-48, OXY-1	2	1 × 10 ¹	1 × 10 ²	1 × 10 ⁵
<i>Klebsiella oxytoca</i> ^N	OXA-48, OXY-1	0.38	1 × 10 ¹	1 × 10 ⁴	1 × 10 ⁷
<i>Enterobacter cloacae</i> ^N	OXA-48, CTX-M-group 1, AmpC	24	1 × 10 ¹	1 × 10 ¹	1 × 10 ²
<i>Enterobacter cloacae</i> ^N	OXA-48, CTX-M-group 1, AmpC	1.5	1 × 10 ¹	1 × 10 ²	1 × 10 ⁵
<i>Enterobacter aerogenes</i> ^N	OXA-48, AmpC	6	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁴
<i>Enterobacter aerogenes</i> ^N	OXA-48, AmpC	2	1 × 10 ¹	1 × 10 ²	1 × 10 ⁵
<i>Citrobacter werkmanii</i> ^B	OXA-48	8	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
<i>Citrobacter werkmanii</i> ^N	OXA-48	0.5	1 × 10 ¹	1 × 10 ²	> 1 × 10 ⁷
<i>Raoultella ornithinolytica</i> ^B	OXA-48	0.5	1 × 10 ¹	1 × 10 ²	> 1 × 10 ⁷
<i>Raoultella planticola</i> ^B	OXA-48	32	1 × 10 ¹	1 × 10 ¹	1 × 10 ³
<i>Pluralibacter gergoviae</i> ^B	OXA-48	8	1 × 10 ¹	1 × 10 ¹	1 × 10 ⁵
Ambler class A carbapenemases (n = 11)					
<i>Klebsiella pneumoniae</i> ^B	KPC-2, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	8	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	KPC-2, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	KPC-2, CTX-M-group 1, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^B	KPC-3, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Escherichia coli</i> ^N	KPC-2, TEM-1	8	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Enterobacter cloacae</i> ^N	IMI-1, AmpC	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
Ambler class B carbapenemases (n = 11)					
<i>Escherichia coli</i> ^N	NDM-1, CTX-M-group 1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Escherichia coli</i> ^N	NDM-1	16	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Escherichia coli</i> ^N	NDM-1, DHA-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Escherichia coli</i> ^B	VIM-1	8	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Escherichia coli</i> ^N	VIM-1	6	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^B	NDM-1, CTX-M-group 1, SHV-1	4	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	NDM-1, CTX-M-group 1, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	NDM-1, CTX-M-group 1, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella pneumoniae</i> ^N	NDM-1, CTX-M-group 1, SHV-1	>32	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ¹
<i>Enterobacter cloacae</i> ^N	VIM-1, AmpC	3	1 × 10 ¹	>1 × 10 ⁷	1 × 10 ⁴
<i>Citrobacter freundii</i> ^N	VIM-1, AmpC	1	1 × 10 ¹	>1 × 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)					
<i>Klebsiella pneumoniae</i> ^{N,1}	CTX-M-group 1, SHV-1	1	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ⁷
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	1.5	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ⁶
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	0.75	1 × 10 ¹	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	2	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ⁶
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	0.38	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	0.38	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	0.38	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	1	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ⁶
<i>Klebsiella pneumoniae</i> ^{N,1}	DHA-1, SHV-1	1	1 × 10 ¹	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^{N,1}	DHA-1, SHV-1	0.38	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^{N,1}	CMY-2	0.5	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^{B,2}	AmpC hyperproduction	0.75	1 × 10 ¹	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^{N,2}	AmpC hyperproduction	0.5	1 × 10 ¹	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	6	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ³
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	3	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ⁵
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	2	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ⁶
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	0.5	1 × 10 ¹	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	0.5	1 × 10 ¹	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction, CTX-M-group 9	0.5	1 × 10 ¹	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Enterobacter cloacae</i> ^{B,2}	AmpC hyperproduction	2	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ⁶
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	0.75	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ⁷
<i>Enterobacter cloacae</i> ^{N,2}	AmpC hyperproduction	0.38	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Citrobacter freundii</i> ^{B,2}	AmpC hyperproduction	0.75	1 × 10 ¹	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Klebsiella oxytoca</i> ^{N,2}	OXY hyperproduction	0.5	1 × 10 ¹	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Klebsiella oxytoca</i> ^{N,2}	OXY hyperproduction	>32	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ¹
<i>Klebsiella oxytoca</i> ^{N,2}	OXY hyperproduction	12	1 × 10 ¹	> 1 × 10 ⁷	1 × 10 ¹
Non-carbapenemase producers being susceptible to carbapenems (n = 32)					
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.25	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.25	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.25	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	CTX-M-group 1	0.25	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^N	CTX-M-group 1	0.023	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	CTX-M-group 1, <i>mcr</i> -1	0.25	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^N	CTX-M-group 1, <i>mcr</i> -1	0.094	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^N	CTX-M-group 9	0.004	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^N	CTX-M-group 9	0.032	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^N	CTX-M-group 9	0.006	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^N	CTX-M-group 9	0.032	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	SHV-12	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Klebsiella pneumoniae</i> ^B	CTX-M-group 1	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Klebsiella pneumoniae</i> ^B	CTX-M-group 1	0.25	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Klebsiella pneumoniae</i> ^N	SHV-5	0.032	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Proteus mirabilis</i> ^N	CTX-M-group 1	0.008	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Enterobacter aerogenes</i> ^B	TEM-24	0.25	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	AmpC hyperproduction	0.25	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^N	AmpC hyperproduction	0.023	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	CMY-4	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	CMY-4	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Enterobacter cloacae</i> ^N	AmpC hyperproduction	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Citrobacter freundii</i> ^N	AmpC hyperproduction	0.19	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Citrobacter freundii</i> ^N	AmpC hyperproduction	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Morganella morganii</i> ^N	AmpC hyperproduction	0.012	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Citrobacter koseri</i> ^B	DHA-1	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Proteus mirabilis</i> ^B	CMY-16	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷
<i>Escherichia coli</i> ^B	ATCC 29522	0.125	> 1 × 10 ⁷	> 1 × 10 ⁷	> 1 × 10 ⁷

ETP, ertapenem; CFU counts are considered as negative results with a cut off values set at 1 × 10³ CFU/plate for calculation of sensitivity and with a cut off value at > 1 × 10⁷ CFU/plate for calculation of specificity; ¹Reduced susceptibility to ertapenem due to porin deficiency; ²Reduced 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 (10² CFU/mL [10¹ 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 (10² CFU/plate) and a high inoculum (10⁴ 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
Sensitivity (%)	100	56.3	43.6	92.7
Sensitivity class D carbapenemase	100	93.9	21.2	93.9
Sensitivity 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.

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