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CHROMagar™ ESBL/mSuperCARBA bi-plate medium for detection of ESBL- and carbapenemase-producing *Enterobacteriaceae* from spiked stools

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ABSTRACT

The recently released CHROMagar™ ESBL/CHROMagar™ mSuperCARBA bi-plate medium was evaluated for the detection of ESBL- and carbapenemase-producing *Enterobacteriaceae*. Spiked stools were used to mimic *in vivo* stool colonization. Two-hundred enterobacterial isolates were tested. Respective sensitivities of 93.9% and 97.8% were obtained for the detection of ESBL and carbapenemase producers.

Resistance to broad-spectrum cephalosporins and subsequently to carbapenems spreads rapidly among *Enterobacteriaceae* at an alarming rate, resulting in both nosocomial and community-acquired infections primarily due to the production of extended-spectrum β-lactamases (ESBLs) and/or carbapenemases (Pitout and Laupland, 2008; Rood and Li, 2017). Targeted surveillance of high-risk patients and screening are essential to prevent outbreaks of nosocomial infections by these organisms. In *Enterobacteriaceae*, clinically relevant carbapenemases belong to the Ambler class A (KPC-type), Ambler class B metallo-β-lactamases (NDM, VIM and IMP), and Ambler class D enzymes (OXA-48-like) (Nordmann et al., 2012a). The level of resistance to carbapenems conferred by these carbapenemase producers may vary widely, making their detection difficult when just based on their *in vitro* susceptibility profile (Landman et al., 2010). ESBLs are enzymes that mediate resistance to penicillins, extended-spectrum third-generation cephalosporins (3GC), and monobactams. ESBL-producing *Enterobacteriaceae* appeared in the 1980s and have since become highly prevalent in nosocomial infections, mainly due to *Escherichia coli* and *Klebsiella* spp. ChromID ESBL (bioMérieux) and CHROMagar™ ESBL (CHROMagar, Paris, France) are very useful for the detection of ESBL enterobacterial producers; nevertheless, their ability to detect isolates producing OXA-48–like carbapenemase alone is limited (Carrer et al., 2010; Hornsey et al., 2013). Consequently, the detection of all carbapenemase-producing *Enterobacteriaceae* (CPE), including OXA-48–like producers, is based on the inoculation of at least 2 media able to efficiently identify i) all types of carbapenemases with the exception of OXA-48–like enzymes (e.g., ChromID® CARBA medium, bioMérieux) and ii) specifically OXA-48–like producers (e.g., ChromID® OXA-48 medium, bioMérieux). These 2 media are now marketed in a single bi-plate, the ChromID® CARBA SMART medium that has been reported to efficiently detect all CPEs. However, this medium is designed for the screening of CPEs and not for ESBL producers.

Another medium had already been developed for the detection of all CPEs, including OXA-48–like carbapenemase producers, the SUPERCARBA medium (Nordmann et al., 2012b). The initial SUPERCARBA medium was further modified to improve its stability and facilitate the presumptive identification of the enterobacterial species by the addition of chromogenic molecules. This improved medium, named mSuperCARBA (CHROMagar Ltd., France), was demonstrated to be reliable for the screening of CPE (Garcia-Fernandez et al., 2017; Garcia-Quintanilla et al., 2018; Genc and Aksu, 2018; Soria Segarra et al., 2018). However, as observed with the ChromID® CARBA SMART medium, this single-plate medium is not suitable for screening of ESBL producers.

Recently, the selective bi-plate medium CHROMagar™ ESBL/CHROMagar™ mSuperCARBA (CHROMagar Ltd., France) was designed for the rapid identification of ESBL-producing and carbapenem-

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Table 1
Sensitivity of detection of the CHROMagar™ ESBL /mSuperCARBA medium from spiked fecal samples.

β-Lactam resistance mechanism	Name of the strain	Species	MIC (μg/mL)			β-Lactamase content	Limit of detection (UFC/mL)[b,c]	
			IPM[a]	ETP	MEM		ESBL	mSuperCARBA
Non–carbapenemase producers								
Acquired case	2 E2	*E. coli*	0.12	0.02	0.02	DHA-1	$>\frac{1\times}{10^4}$	$>1\times10^4$
	2 E3	*E. coli*	0.12	0.12	0.12	ACC-1	$>\frac{1\times}{10^4}$	$>1\times10^4$
	2 E4	*K. pneumoniae*	0.12	0.5	0.12	DHA-2	1×10^2	$>1\times10^4$
	2 E5	*P. mirabilis*	0.25	0.12	0.12	ACC-1	$>\frac{1\times}{10^4}$	$>1\times10^4$
ESBL	2 E6	*E. coli*	0.12	0.12	0.12	CTX-M-1	1×10^2	$>1\times10^4$
	2 E7	*E. coli*	0.12	0.12	0.12	CTX-M-3	1×10^2	$>1\times10^4$
	2 E8	*K. pneumoniae*	0.12	0.12	0.12	CTX-M-3	1×10^2	$>1\times10^4$
	2 E9	*E. coli*	0.12	0.12	0.12	CTX-M-14	1×10^2	$>1\times10^4$
	2 E10	*E. coli*	0.12	0.12	0.12	CTX-M-14	1×10^2	$>1\times10^4$
	2 F1	*K. pneumoniae*	0.12	0.12	0.12	CTX-M-14	1×10^2	$>1\times10^4$
	2 F2	*E. coli*	0.12	0.12	0.12	CTX-M-15	1×10^2	$>1\times10^4$
	2 F3	*E. coli*	0.12	0.12	0.12	CTX-M-15	1×10^2	$>1\times10^4$
	2 F4	*K. pneumoniae*	0.12	0.12	0.12	CTX-M-15	1×10^2	$>1\times10^4$
	2 F5	*K. pneumoniae*	0.12	0.12	0.12	CTX-M-15	1×10^2	$>1\times10^4$
	2 F6	*K. pneumoniae*	0.12	0.12	0.12	CTX-M-15	1×10^2	$>1\times10^4$
	2 F7	*E. cloacae*	0.12	0.12	0.12	CTX-M-15	1×10^2	$>1\times10^4$
	2 F8	*E. cloacae*	0.12	0.12	0.12	VEB-1	1×10^2	$>1\times10^4$
Case + impermeability	2 F9	*E. coli*	16	>32	2	↗↗↗ Case	3×10^2	2×10^2
	CNR 92 10	*E. cloacae*	0.19	1	0.12	↗↗↗ Case	1×10^2	$>1\times10^4$
	CNR 92 J6	*E. cloacae*	0.25	1	0.12	↗↗↗ Case	$>\frac{1\times}{10^4}$	$>1\times10^4$
	2 F10	*E. cloacae*	0.12	1	0.12	↗↗↗ Case	1×10^3	$>1\times10^4$
	2 G1	*E. cloacae*	0.12	1	0.12	↗↗↗ Case	1×10^2	$>1\times10^4$
	2 G2	*E. cloacae*	0.25	4	0.25	↗↗↗ Case	1×10^2	2×10^3
	2 G3	*E. cloacae*	4	1.5	0.75	↗↗↗ Case	5×10^3	$>1\times10^4$
	2 G4	*E. cloacae*	0.19	1.5	0.12	↗↗↗ Case	1×10^2	$>1\times10^4$
	2 G5	*E. cloacae*	0.5	4	0.75	↗↗↗ Case	1×10^2	$>1\times10^4$
	2 G6	*E. cloacae*	1.5	0.75	0.25	↗↗↗ Case	1×10^2	$>1\times10^4$
	2 G7	*E. cloacae*	1.5	2	0.75	↗↗↗ Case	1×10^2	$>1\times10^4$
	2 G8	*E. cloacae*	0.5	1.5	0.38	↗↗↗ Case	1×10^2	$>1\times10^4$
	2 G9	*E. cloacae*	2	4	1.5	↗↗↗ Case	1×10^2	1×10^3
	2 G10	*E. cloacae*	8	>32	4	↗↗↗ Case	1×10^2	4×10^2
	2 H1	*E. cloacae*	0.5	4	0.75	↗↗↗ Case	1×10^2	1×10^2
	2 H2	*E. cloacae*	0.19	3	0.38	↗↗↗ Case	1×10^2	1×10^2
	2 H3	*E. cloacae*	1.5	3	0.75	↗↗↗ Case	1×10^2	$>1\times10^4$
	2 H4	*E. aerogenes*	1	4	0.75	↗↗↗ Case	1×10^2	$>1\times10^4$
	2 H5	*M. morgannii*	1.5	0.02	0.12	↗↗↗ Case	$>\frac{1\times}{10^4}$	$>1\times10^4$
	CNR 87 J10	*H. alvei*	0.25	1	0.09	↗↗↗ Case	$>\frac{1\times}{10^4}$	$>1\times10^4$
	CNR 88 A1	*H. alvei*	0.38	6	0.75	↗↗↗ Case	$>\frac{1\times}{10^4}$	1×10^2
	CNR 87 F7	*S. marcescens*	0.75	0.75	0.19	↗↗↗ Case	1×10^2	1×10^2
	CNR 87 B7	*E. cloacae*	0.25	0.75	0.04	↗↗↗ Case	1×10^2	$>1\times10^4$
ESBL + impermeability	2 H6	*E. coli*	2	4	1	CTX-M-15	1×10^2	1×10^2
	2 H7	*K. pneumoniae*	1	>32	4	CTX-M-15 + SHV-1	1×10^2	1×10^2
	2 H8	*K. pneumoniae*	1.5	>32	4	CTX-M-15 + TEM-1 + SHV-1	1×10^2	1×10^2
	2 H9	*K. pneumoniae*	0.25	1	1	CTX-M-15 + TEM-1 + SHV-1	1×10^2	$>1\times10^4$
	2 H10	*K. pneumoniae*	1.5	>32	6	CTX-M-15 + SHV-11	1×10^2	1×10^2
	2 I1	*K. pneumoniae*	8	>32	4	CTX-M-15 + SHV-28 - TEM-1	1×10^2	1×10^2
	2 I2	*K. pneumoniae*	1	4	1	TEM-1 + SHV-28	1×10^2	1×10^2
	2 I3	*K. pneumoniae*	3	>32	6	CTX-M-15 + TEM-1 + SHV-11	1×10^2	1×10^2
	2 I4	*K. pneumoniae*	0.25	1	1	CTX-M-15 + TEM-1 + SHV-11	1×10^2	1×10^2
	2 I5	*K. pneumoniae*	6	> 32	> 32	CTX-M-15 + TEM-1 + SHV-11	1×10^2	1×10^2
	2 I6	*K. pneumoniae*	0.75	> 32	3	CTX-M-15 + TEM-1 + SHV-12	1×10^2	1×10^2
	2 I7	*K. pneumoniae*	1	24	0.5	CTX-M-15 +TEM-1 + SHV-11	1×10^2	1×10^2
	2 I8	*K. pneumoniae*	2	4	1	CTX-M-15 + TEM-1 + SHV-1 + OXA-1	1×10^2	$>1\times10^4$
Hyper K1 + impermeability	CNR 151 J9	*K. oxytoca*	2	3	0.75	↗↗↗ K1 penicillinase	1×10^2	1×10^2
	CNR 92 J7	*K. oxytoca*	1	2	0.5	↗↗↗ K1 penicillinase	1×10^2	$>1\times10^4$
ESBL + Case + impermeability	2 I9	*E. cloacae*	1.5	6	1	↗↗↗ Case + CTX-M-15	1×10^2	$>1\times10^4$
	2 I10	*E. cloacae*	2	8	1	↗↗↗ Case + CTX-M-15	1×10^2	$>1\times10^4$
	2 J1	*E. cloacae*	3	12	2	↗↗↗ Case + CTX-M-15	1×10^2	$>1\times10^4$
	2 J2	*C. freundii*	1	8	1	↗↗↗ Case +TEM-3	1×10^2	1×10^2
Extended spectrum	2 J3	*K. pneumoniae*	0.5	0.38	0.12	OXA-163	$>1\times$	$>1\times10^4$

Table 1 *(continued)*

β-Lactam resistance mechanism	Name of the strain		Species	MIC (μg/mL)			β-Lactamase content	Limit of detection (UFC/mL)[b,c]	
				IPM[a]	ETP	MEM		ESBL	mSuperCARBA
oxacillinases									
	2	J4	*E. cloacae*	0.5	2	0.19	OXA-163	2×10^2	$> 1 \times 10^4$
	2	J5	*S. marcescens*	0.5	0.75	0.19	OXA-405	$> 1 \times 10^4$	$> 1 \times 10^4$
Carbapenemase producers									
	1	F1	*E. coli*	1	>32	3	KPC-2	1×10^2	1×10^2
	1	F2	*E. coli*	0.5	0.5	0.5	KPC-2	1×10^2	1×10^2
	1	F3	*E. coli*	2	1.5	1	KPC-2 + TEM-1 + OXA-9	1×10^2	1×10^2
	1	F4	*E. coli*	4	4	2	KPC-2 + CTX-M-9 + TEM-1	1×10^2	4×10^2
	1	F5	*K. pneumoniae*	16	24	32	KPC-2 + SHV-11 + TEM-1 + CTX-M-2	1×10^2	1×10^2
	1	F6	*K. pneumoniae*	>32	>32	>32	KPC-2 + SHV-11 + TEM-1 + CTX-M-2 + OXA-9	1×10^2	1×10^2
	1	F7	*K. pneumoniae*	16	>32	>32	KPC-2 + SHV-11 + CTX-M-15	1×10^2	1×10^2
	1	F8	*K. pneumoniae*	4	4	32	KPC-2 + TEM-1 + SHV-1 + CTXM-15	1×10^2	1×10^2
	1	F9	*K. pneumoniae*	4	24	2	KPC-2 + SHV-11 + TEM-1 + SHV-12 + OXA-9	1×10^2	1×10^2
	1	F10	*K. pneumoniae*	>32	>32	>32	KPC-2 + SHV-11	1×10^2	1×10^2
	1	G1	*K. pneumoniae*	4	6	8	KPC-2 + SHV-11 + TEM-1	1×10^2	1×10^2
KPC-type	1	G2	*K. pneumoniae*	4	>32	8	KPC-3 + TEM-1 + SHV-1 + CTX-M-15 + OXA-9	1×10^2	1×10^2
	1	G3	*K. pneumoniae*	8	>32	8	KPC-3 + SHV-11 + OXA-9 + TEM-1	1×10^2	1×10^2
	1	G4	*K. ozoenae*	>32	>32	2	KPC-3 + OXA-9 + TEM-1	$> 1 \times 10^4$	2×10^2
	1	G5	*E. cloacae*	1	1.5	0.75	KPC-2	1×10^2	1×10^2
	1	G6	*E. cloacae*	24	>32	16	KPC-2 + TEM-1	1×10^2	1×10^2
	1	G7	*E. cloacae*	4	6	2	KPC-2 + TEM-1 + OXA-1	1×10^2	1×10^2
	1	G8	*E. cloacae*	2	4	1.5	KPC-2 + TEM-1 + SHV-11	1×10^2	1×10^2
	1	G9	*E. cloacae*	2	2	1	KPC-2 + TEM-3	1×10^2	1×10^2
	1	G10	*C. freundii*	8	1.5	3	KPC-2 + TEM-1	$> 1 \times 10^4$	1×10^2
	1	I1	*S. marcecens*	>32	>32	>32	KPC-2 + TEM-1 + SHV-12	1×10^2	1×10^2
	1	I2	*S. marcecens*	>32	>32	>32	KPC-2 + TEM-1	1×10^2	1×10^2
	1	I3	*E. cloacae*	>32	8	4	IMI-1	$> 1 \times 10^4$	1×10^2
	1	I4	*E. asburiae*	>32	>32	>32	IMI-2	$> 1 \times 10^4$	1×10^2
IMI-type	1	I5	*E. asburiae*	>32	8	2	IMI-2	$> 1 \times 10^4$	3×10^2
NMC-A	1	I6	*E. cloacae*	>32	1,5	0,75	NmcA	$> 1 \times 10^4$	3×10^3
	1	I7	*S. marcescens*	>32	8	8	Sme-1	$> 1 \times 10^4$	$> 1 \times 10^4$
SME-type	1	I8	*S. marcescens*	>32	8	8	Sme-2	$> 1 \times 10^4$	1×10^2
GES-type	1	I9	*E.cloacae*	>32	>32	>32	GES-5	1×10^2	1×10^2
FRI-1	1	I10	*E. cloacae*	>32	>32	16	FRI-1	1×10^2	1×10^2
	1	A1	*E. coli*	1	3	1	NDM-1 + OXA-1 + OXA-10 + CMY-16 + TEM-1	1×10^2	1×10^2
	1	A2	*E. coli*	3	3	2	NDM-1 + OXA-1 + TEM-1	1×10^2	1×10^2
	1	A3	*E. coli*	6	32	16	NDM-1 + CTX-M-15 + TEM-1	1×10^2	1×10^2
	1	A4	*E. coli*	4	>32	8	NDM-1 + OXA-1 + OXA-2 + CTX-M-15 + TEM-1	1×10^2	1×10^2
	1	A5	*E. coli*	16	>32	16	NDM-1 + CTX-M-15 + TEM-1	1×10^2	1×10^2
	1	A6	*E. coli*	>32	>32	>32	NDM-4 + CTX-M-15 + OXA-1	1×10^2	1×10^2
	1	A7	*E. coli*	>32	>32	>32	NDM-4 + CTX-M-15 + CMY-6	1×10^2	1×10^2
	1	A8	*E. coli*	>32	>32	>32	NDM-5 + TEM-1 + CTX-M-15	1×10^2	1×10^2
	1	A9	*E. coli*	6	32	8	NDM-6 + CTX-M-15 + OXA-1	1×10^2	1×10^2
	1	A10	*E. coli*	4	16	3	NDM-7	1×10^2	1×10^2
	1	B1	*K. pneumoniae*	2	8	3	NDM-1 + CTX-M-15 + SHV-11 + OXA-1	1×10^2	1×10^2
NDM-type	1	B2	*K. pneumoniae*	>32	>32	>32	NDM-1 + CTX-M-15 + CMY-4 + OXA-1	1×10^2	1×10^2
	1	B3	*K. pneumoniae*	>32	>32	>32	NDM-1 + CTX-M-15 + OXA-1 + OXA-9 + TEM-1 + SHV-28 + SHV-11	1×10^2	1×10^2
	1	B4	*K. pneumoniae*	1.5	6	2	NDM-1 + OXA-1 + SHV-11	1×10^2	1×10^2
	1	B5	*K. pneumoniae*	1	8	4	NDM-1 + OXA-1 + CTX-M-15 + TEM-1 + SHV-28 + OXA-9 + CMY-6	1×10^2	1×10^2
	1	B6	*K. pneumoniae*	1.5	8	1.5	NDM-1 + TEM-1 + CTX-M-15 + SHV-12 + OXA-9	1×10^2	1×10^2
	1	B7	*K. pneumoniae*	4	8	16	NDM-1 + TEM-1 + CTX-M-15 + SHV-12 + OXA-9	1×10^2	1×10^2
	1	B8	*K. pneumoniae*	2	>32	4	NDM-1 + TEM-1 + CTX-M-15 + SHV-11 + OXA-1	1×10^2	1×10^2
	1	B9	*P. stuartii*	12	0.38	1.5	NDM-1 + OXA-1 + CMY-6 + TEM-1	1×10^2	$> 1 \times 10^4$
	1	B10	*P. rettgeri*	3	0.5	1.5	NDM-1 + CTX-M-15	1×10^2	1×10^2
	1	C1	*Salmonella enterica*	4	6	3	NDM-1 + CTX-M-15 + TEM-1 + OXA-1 + OXA-9 + OXA-10	5×10^2	2×10^2
	1	C2	*E. coli*	1.5	0.38	0.5	VIM-1 + CTX-M-3	1×10^2	$> 1 \times 10^4$
	1	C3	*E. coli*	3	1.5	1	VIM-1 + CMY-13	2×10^3	6×10^2
VIM-type	1	C4	*E. coli*	8	4	3	VIM-4	1×10^2	1×10^2
	1	C5	*K. pneumoniae*	>32	>32	>32	VIM-1 + SHV-5	1×10^2	1×10^2
	1	C6	*K. pneumoniae*	>32	>32	>32	VIM-1	1×10^2	1×10^2

(continued on next page)

Table 1 *(continued)*

β-Lactam resistance mechanism	Name of the strain	Species	MIC (μg/mL)			β-Lactamase content	Limit of detection (UFC/mL)[b,c]	
			IPM[a]	ETP	MEM		ESBL	mSuperCARBA
	1 C7	*K. pneumoniae*	>32	>32	>32	VIM-1 + SHV-12	1×10^2	1×10^2
	1 C8	*K. pneumoniae*	>32	>32	>32	VIM-1	1×10^2	1×10^2
	1 C9	*K. pneumoniae*	4	2	2	VIM-1 + SHV-5	1×10^2	1×10^2
	1 C10	*K. pneumoniae*	>32	>32	>32	VIM-1 + TEM-1 + SHV-5	1×10^2	1×10^2
	1 D1	*K. pneumoniae*	>32	>32	>32	VIM-1 + SHV-5	1×10^2	1×10^2
	1 D2	*K. pneumoniae*	1	0.5	1	VIM-1 + CTX-M-3	1×10^2	1×10^2
	1 D3	*K. pneumoniae*	0.5	4	0.38	VIM-1 + SHV-5	1×10^2	1×10^2
	1 D4	*K. pneumoniae*	8	16	4	VIM-19 + CTX-M-3 + TEM-1 + SHV-1	1×10^2	1×10^2
	1 D5	*E.cloacae*	1	0.38	0.5	VIM-1 + SHV-70	1×10^2	3×10^2
	1 D6	*E. cloacae*	3	2	1	VIM-4 + CTX-M-15 + TEM-1 + SHV-31	1×10^2	1×10^2
	1 D7	*C. freundii*	2	2	0.75	VIM-2 + TEM-1 +	1×10^2	1×10^2
	1 D8	*C. freundii*	1.5	4	0.5	VIM-2 + TEM-1 + OXA-9 + OXA-10	1×10^2	1×10^2
	1 D9	*E. coli*	0.5	3	0.5	IMP-1	1×10^2	1×10^2
	1 D10	*E. coli*	6	8	3	IMP-8 + SHV -12	1×10^2	1×10^2
	1 E1	*K. pneumoniae*	1.5	3	1	IMP-1	1×10^2	1×10^2
	1 E2	*K. pneumoniae*	8	3	2	IMP-1 + TEM-15	1×10^2	1×10^2
	1 E3	*K. pneumoniae*	1.5	4	2	IMP-1 + TEM-15 + CTX-M-15	1×10^2	1×10^2
IMP-type	1 E4	*K. pneumoniae*	1	2	8	IMP-1 + SHV-5	1×10^2	1×10^2
	1 E5	*K. pneumoniae*	1	1	0.5	IMP-8	1×10^2	1×10^2
	1 E6	*K. pneumoniae*	0.5	0.5	0.5	IMP-8 + SHV -12	1×10^2	1×10^2
	1 E7	*E. cloacae*	1.5	1	1	IMP-8	1×10^2	1×10^2
	1 E8	*E. cloacae*	0.75	0.5	0.5	IMP-8 + SHV-12	1×10^2	1×10^2
	1 E9	*S. marscecens*	8	>32	2	IMP-11	1×10^2	1×10^2
GIM-type	1 E10	*E. cloacae*	2	>32	6	GIM-1	1×10^2	1×10^2
	2 A1	*E. coli*	3	16	1	OXA-48 + CTX-M-15	1×10^2	1×10^2
	2 A2	*E. coli*	0.5	0.75	0.12	OXA-48 + CTX-M-15	1×10^2	1×10^2
	2 A3	*E. coli*	0.38	1.5	0.19	OXA-48 + CTX-M-15	1×10^2	1×10^2
	2 A4	*E. coli*	0.25	0.5	0.19	OXA-48 + CTX-M-24 + TEM-1	1×10^2	1×10^2
	2 A5	*E. coli*	0.75	1	0.19	OXA-48 + CTX-M-24 + TEM-1	1×10^2	1×10^2
	2 A6	*E. coli*	0,5	1	0.25	OXA-48 + CTX-M-15	1×10^2	2×10^2
	2 A7	*K. pneumoniae*	0.5	2	0.5	OXA-48	2×10^3	1×10^2
	2 A8	*K. pneumoniae*	0.38	1	0.5	OXA-48 + TEM-1	$>1 \times 10^4$	1×10^2
	2 A9	*K. pneumoniae*	2	3	2	OXA-48 + CTX-M-15	1×10^2	1×10^2
	2 A10	*K. pneumoniae*	1	4	1	OXA-48	$>1 \times 10^4$	1×10^2
OXA-48	2 B1	*K. pneumoniae*	1	4	1	OXA-48	$>1 \times 10^4$	1×10^2
	2 B2	*K. pneumoniae*	>32	>32	>32	OXA-48	6×10^3	1×10^2
	2 B3	*K. pneumoniae*	0.5	0.75	0.25	OXA-48 + SHV-11	$>1 \times 10^4$	1×10^2
	2 B4	*K. pneumoniae*	0,5	>32	1,5	OXA-48 + CTX-M-15 + TEM -1	1×10^2	1×10^2
	2 B5	*K. pneumoniae*	0.75	16	1	OXA-48 + CTX-M-15 + TEM-1	1×10^2	1×10^2
	2 B6	*E. cloacae*	0.5	2	0.5	OXA-48 + TEM-1 + CTX-M-15 + OXA-1	1×10^2	1×10^2
	2 B7	*E. cloacae*	1	16	1.5	OXA-48 + TEM-1 + CTX-M-15 + OXA-1	1×10^2	1×10^2
	2 B8	*E. cloacae*	2	8	1	OXA-48 + SHV-5	1×10^2	1×10^2
	2 B9	*C. koseri*	0.38	2	0.38	OXA-48	1×10^3	1×10^2
	2 B10	*C. koseri*	0.75	2	0.38	OXA-48 + TEM-1	1×10^2	1×10^2
	2 C1	*C. freundii*	0.75	1,5	0.38	OXA-48 + SHV-12 + TEM-1	1×10^2	1×10^2
	2 C2	*K. pneumoniae*	4	8	1	OXA-162 + TEM-1 + SHV-11	1×10^2	1×10^2
OXA-162	CNR 67 F9	*K. pneumoniae*	0.38	8	0.5	OXA-162	$>1 \times 10^4$	1×10^2
	2 C3	*E. coli*	0.38	1.5	0.19	OXA-181	1×10^3	1×10^2
	2 C4	*E. coli*	0.5	1.5	0.25	OXA-181	$>1 \times 10^4$	1×10^4
	CNR 51 E10	*E. coli*	0.38	0.38	0.12	OXA-181	1×10^2	1×10^4
	CNR 59 F5	*E. coli*	0.38	1.5	0.12	OXA-181	1×10^2	1×10^2
OXA-181	CNR 61 C6	*E. coli*	0.25	1	0.12	OXA-181	1×10^2	1×10^2
	CNR 61 D1	*E. coli*	0.25	1.5	0.12	OXA-181	1×10^2	1×10^2
	CNR 64 C4	*E. coli*	>32	>32	12	OXA-181	1×10^2	1×10^2
	2 C9	*K. pneumoniae*	0.5	2	0.5	OXA-181 + SHV-11 + CTXM-15 + OXA-1	1×10^2	1×10^2
	CNR 58 J8	*K. pneumoniae*	0.38	1.5	0.38	OXA-181	1×10^2	1×10^2
	2 D2	*K. pneumoniae*	0.5	2	0.5	OXA-204 + CMY-4	1×10^2	1×10^2
	2 D3	*E. coli*	0.38	0.19	0,094	OXA-204 + CMY-2 + CTX-M-15 + OXA-1	1×10^2	1×10^2
OXA-204	2 D4	*E. coli*	0,5	2	0.25	OXA-204 + CMY-4+ CTX-M-15 + OXA-1	1×10^2	1×10^4
	2 D5	*E. coli*	0,5	2	0.38	OXA-204 + CMY-4 + CTX-M-15	1×10^2	1×10^3
	2 D6	*K. pneumoniae*	0,5	16	0.75	OXA-204 + SHV-28 + TEM-1 + CTX-M-15	1×10^2	1×10^2
OXA-232	2 D7	*E. coli*	>32	>32	>32	OXA-232 + CTX-M-15 + OXA-1	1×10^2	1×10^2

resistant *Enterobacteriaceae*. The aim of the present study was to evaluate the performance of this bi-plate medium using spiked stool samples with a collection of well-characterized CPE and ESBL producers at the molecular level.

Two hundred and three enterobacterial isolates were tested (Table 1), including 137 carbapenemase producers [30 Ambler class A (KPC, Sme, IMI, GES-5, FRI-1), 50 Ambler class B (IMP, VIM, GIM-1, NDM-type), 43 Ambler class D (OXA-48-type and OXA-372), 14 multiple carbapenemase producers] and 62 non–carbapenemase producers [ESBL with or without impaired outer-membrane (n=26), cephalosporinase producers with or without an altered outer membrane (n=27), concomitant ESBL and overexpressed cephalosporinase producers (n=4), K1 penicillinase or extended-spectrum oxacillinases producers (n=5)]. The β-lactamase content of all these isolates was characterized by endpoint PCR followed by Sanger sequencing or by whole genome sequencing. In addition, the Carba NP test was also performed on all isolates as previously described (Dortet et al., 2015). Fully susceptible strains of *E. coli* ATCC 25922 and *K. pneumoniae* CIP53153, and ESBL-producing strains of *E. coli* CIP103983 (TEM-4) and *K. pneumoniae* ATCC 700603 (SHV-18) were used as controls (not included in the table of results).

CHROMagar™ ESBL/mSuperCARBA is a ready-to-use bi-plate with a shelf life of 60 days at +4 °C (+2 °C to +12 °C). Bacterial suspensions with a 0.5 McFarland (inoculum of ~5×10^7 to 10^8 CFU/mL) of the different isolates were serially diluted (10-fold dilutions) in water. Spiked fecal samples were prepared by adding 100 μL of each dilution to 900 μL of fecal suspension obtained by resuspending 4 g of freshly pooled feces from 4 healthy volunteers in 40 mL of distilled water, as previously described (Naas et al., 2011). A fecal suspension without spiking was used as negative control. The lowest detection limits were determined by plating 10 μL of each spiked fecal samples on CHROMagar™ ESBL/mSuperCARBA medium. Viable bacteria were counted after 24 h of culture at 37 °C. The lowest detection limit (LOD) corresponds to the minimum number of bacteria that must be present in the sample to obtain a growth on the selective medium. In order to compare both selective media, the cutoff value was arbitrarily set at > 1×10^4 CFU/mL. A minimal concentration greater than 1×10^4 CFU/mL was considered as nondetection, and sensitivity and specificity values were determined accordingly (Table 1).

Considering the 62 non–carbapenemase producers, all ESBL producers with (n=13) or without an altered outer membrane (n=13) grew on the CHROMagar™ ESBL medium, while 75% of the enterobacterial isolates with an acquired cephalosporinase did not grow with the exception of 1 DHA-2–producing *K. pneumoniae* isolate (considered as a false positive). However, 19/23 isolates with overexpression of the cephalosporinase associated with decreased altered outer membrane grew on the CHROMagar™ ESBL medium. They were falsely considered as potential ESBL producers. Only 1e of the 3 extended-spectrum oxacillinase producers grew on the CHROMagar™ ESBL medium (thus giving 2 false negatives). Finally, the 2 penicillinase K1 overexpressing *K. oxytoca* were also falsely detected as ESBL producers. Overall, the sensitivity of the CHROMagar™ ESBL medium for the detection of true ESBL producers was 93.9% [95% confidence interval (CI95) = 78.4–98.9%] in this study. Due to the high number of isolates with overexpressed cephalosporinase associated with decreased outer membrane permeability and the absence of isolates with wild-type phenotypes, the specificity of the CHROMagar™ ESBL medium was low (24.1%). Despite this limitation in the number of susceptible isolates tested, several studies have previously demonstrated that this CHROMagar™ ESBL medium is useful for the efficient detection of ESBL producers (Grohs et al., 2013; Saito et al., 2010).

Among the 137 CPEs, all but 3 were cultured on the CHROMagar™ mSuperCARBA medium. One Sme-1–producing *Serratia marcescens*, one NDM-1–producing *Providencia stuartii*, and one VIM-1–producing

Table 1 *(continued)*

β-Lactam resistance mechanism	Name of the strain	Species	MIC (μg/mL)			β-Lactamase content	Limit of detection (UFC/mL)[b,c]	
			IPM[a]	ETP	MEM		ESBL	mSuperCARBA
OXA-244	2 D8	*K. pneumoniae*	3	>32	12	OXA-232 +SHV-1 + TEM-1 + CTX-M-15 + OXA-1	1×10^2	1×10^2
	CNR 68 B2	*E. coli*				OXA-232	$\underline{> 1 \times 10^4}$	1×10^2
	2 D9	*E. coli*	0.5	2	0.5	OXA-244 + TEM-1 + CMY-2	$\underline{> 1 \times 10^4}$	1×10^2
	2 D10	*E. coli*	0.5	1.5	0.5	OXA-244 + TEM-1 + CMY-2	1×10^2	1×10^2
OXA-372	2 J6	*C. freundii*	3	2	0.5	OXA-372 + CMY-135 + OXA-10 + MOX-9	1×10^2	1×10^2
Multiple carbapenemases	2 C5	*K. pneumoniae*	3	>32	4	OXA-181 + SHV-11 + TEM-1 + CTX-M-15 + NDM-1 + OXA-1	1×10^2	1×10^2
	2 C6	*K. pneumoniae*	>32	>32	>32	OXA-181 + SHV-27 + CTX-M-15 + TEM-1 + NDM-1 + OXA-1	1×10^2	1×10^2
	2 C7	*K. pneumoniae*	>32	>32	>32	OXA-181 + SHV-11 + CTX-M-15 + NDM-1 + OXA-1	1×10^2	1×10^2
	2 C8	*K. pneumoniae*	16	>32	>32	OXA-181 + SHV-11 + TEM-1 + CTX-M-15 + NDM-1 + OXA-9	1×10^2	1×10^2
	2 C10	*K.pneumoniae*	0.5	2	0.5	OXA-181 + NDM-1 + SHV-2 + CTX-M-15 + OXA-1	1×10^2	1×10^2
	2 D1	*C.freundii*	>32	>32	16	OXA-181 + NDM-1 + OXA-1 + OXA-9 + OXA-10 + CTX-M-15 + TEM-1	1×10^2	1×10^2
	CNR 60 A6	*E. coli*	16	>32	16	NDM-1 + OXA-48	1×10^2	1×10^2
	CNR 63 C5	*E. coli*	2	>32	3	NDM-1 + OXA-48	1×10^2	1×10^2
	CNR 67 D2	*E. coli*	1.5	>32	3	NDM-1 + OXA-48	1×10^2	1×10^2
	CNR 22 F9	*K. pneumoniae*	4	>32	8	NDM-1 + OXA-232	1×10^2	1×10^2
	CNR 46 A8	*E. coli*	2	>32	6	NDM-1 + OXA-232	1×10^2	1×10^2
	CNR 28 10	*K. pneumoniae*	>32	>32	>32	NDM-5 + OXA-232	1×10^2	1×10^2
	CNR 51 A9	*E. coli*	0.75	1	0.38	NDM-1 + VIM-2	1×10^2	1×10^2
	CNR 45 J4	*E. cloacae*	1	3	0.75	VIM-4 + OXA-48	1×10^2	1×10^2

[a] IPM = imipenem; ETP = ertapenem; MEM = meropenem; ↗↗↗ Case = overexpressed cephalosporinase.
[b] One ml of stools contains 100 mg of stool.
[c] Underlined CFU counts are considered as negative results (cutoff values set at ≥1×10^4 CFU/mL).

E. coli (Table 1) did not grow on the "carba side of the plate"; however, NDM-1–producing *P. stuartii* and VIM-1–producing *E. coli* grew on the ESBL side of the plate. As previously described with the SUPERCARBA medium, all OXA-48–like producers, known to have a lower carbapenemase activity compared to other carbapenemases (KPC, NDM, VIM, IMP), were detected irrespective of the production of an associated ESBL (Nordmann et al., 2012b). Despite high MICs of carbapenems, most (15/23) non–carbapenemase producers with overexpression of the cephalosporinase associated with decreased outer membrane permeability did not culture on mSuperCARBA medium (Table 1). Overall, the sensitivity of the CHROMagar™ mSuperCARBA medium for the detection carbapenemase producers was 97.8% [CI95 = 93.2–99.4%] in this study. The specificity was 66.1% [CI95 = 52.9–77.4%], which corresponds mainly to the growth of isolates with reduced susceptibility to carbapenems due to ESBL production and/or AmpC overexpression plus impermeability of the outer membrane (Table 1). Due to the low specificity of both sides of the bi-plate, confirmatory tests for ESBLs or carbapenemases detection should be undertaken on any growth. This is a major drawback that will increase the turnaround time and costs if a rapid test is implemented as a confirmation test. However, this low specificity is mostly due to the design of the strains collection and might be better from clinical samples.

Our results suggest that the CHROMagar™ ESBL/mSuperCARBA bi-plate might be a reliable tool for the concomitant screening of patients colonized with ESBL and/or CPE. Currently, in France, hospitalized patients who have traveled abroad are screened for the presence of CPE and ESBL-producing Enterobacteriaceae. Different infection-control measures are implemented and maintained according to the screening results (Fournier et al., 2018): i) standard precautions [no ESBL producer, no CPE, and no glycopeptide-resistant *Enterococcus faecium* (GRE)], ii) contact precautions without cohorting (presence of ESBL producer only), and iii) 'extensively drug-resistant bacteria' (eXDR) control program that includes dedicated staff, isolation of carrier and even cohorting, if possible, and active contact tracing and screening. This active screening of eXDR involves the inoculation of 3 to 4 different media including 1 for the detection of ESBL producers, 1 (bi-plate) or 2 media for the detection of CPE, and 1 for the screening of GRE. In the field of laboratory automation in clinical microbiology (Croxatto and Greub, 2017; Lina and Greub, 2016), the panel of media that can be loaded onto the sorter of the inoculation system is limited (Croxatto et al., 2016). Accordingly, the implementation of CHROMAGAR™ ESBL/mSuperCARBA bi-plate might be an advantage since this medium can replace 2 or 3 of the currently used media for the screening of ESBL and CPE producers. The cost of these bi-plates will be approximately 3.25€/plate.

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Transparency declaration

None to declare.

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