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Comparative evaluation of a novel chromogenic medium (chromID OXA-48) for detection of OXA-48 producing Enterobacteriaceae☆,☆☆

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Comparative evaluation of the recently developed chromogenic culture medium chromID OXA-48 (bioMérieux) with chromID CARBA (bioMérieux) and SUPERCARBA showed that chromID OXA-48 and SUPERCARBA media have the highest sensitivity for detection of OXA-48 producing Enterobacteriaceae (91% and 93%) comparatively to chromID CARBA (21 %). The chromID OXA-48 has the highest specificity, with 100%, as compared to 53% and 68% for the SUPERCARBA and chromID CARBA media for detecting those OXA-48 producers.

Resistance to carbapenems is spreading among Enterobacteriaceae, and carbapenemase-producing Enterobacteriaceae (CPE) is increasingly reported worldwide (Castanheira et al., 2011; Nordmann et al., 2011, 2012a, 2012b, 2012c; Poirel et al., 2012). Adequate preventive measures and efficient screening are needed for an active surveillance to prevent outbreaks of nosocomial infections by these organisms Adler et al., 2011. The main carbapenemases identified in Enterobacteriaceae belong either to the Ambler class A (KPC-type) hydrolysing all β-lactams except cephamycins, the Ambler class B (NDM, VIM and IMP) which are zinc-dependent metallo-β-lactamases (MBL) hydrolysing all β-lactams except aztreonam, and the Ambler class D enzymes (OXA-48-like) hydrolysing carbapenems but weakly and not at all broad-spectrum cephalosporins (Nordmann et al., 2011, 2012a, 2012b, 2012c). The level of resistance to carbapenems conferred by those carbapenemase may vary significantly, making their detection difficult when based on in vitro susceptibility values (Landman et al., 2010). OXA-48 producers currently represent a worrisome threat at least in North African countries, the Middle East, Turkey, the Indian subcontinent, and Europe (Poirel et al., 2012). Moreover, the spread of MBL and KPC producers has created an urgent need for reliable screening techniques for detection of any type of carbapenemase producers (Nordmann et al., 2012b; Vatopoulos, 2008; Vrioni et al., 2012; Wilkinson et al., 2012). The chromogenic, carbapenem-containing chromID CARBA medium and the recently developed SUPERCARBA medium which contains ertapenem, cloxacillin and zinc sulfate have been specifically developed for the detection of CPE (Nordmann et al., 2012b). However the chromID CARBA medium does not detect well OXA-48 producers. This is the reason why the chromID OXA-48 agar was developed intended to screen efficiently OXA-48 carbapenemase-producing Enterobacteriaceae. The aim of the present study was to compare the performance of chromID OXA-48 medium with that of the SUPERCARBA medium (Nordmann et al., 2012b) and that of the commercially-available chromID CARBA selective medium for the detection of OXA-48 producing Enterobacteriaceae.

One hundred seventeen enterobacterial isolates were tested, including OXA-48 producers (n = 53), OXA-48 variants with carbapenemase properties (OXA-162, OXA-181, OXA-204, and OXA-232) (n = 4), KPC-producers (n = 10), MBL-producers (n = 10), non-carbapenemase producers with reduced susceptibility or resistance to carbapenems (AmpC-overproducers, ESBL-producers combining porin deficiency) (n = 20), and carbapenem susceptible isolates (n = 20). The β-lactamase content of all these isolates has been characterized at the molecular level (Table 1). Strains with reduced susceptibility to ertapenem due to an overexpressed AmpC or an ESBL and/or porin deficiency had been previously characterized (inhibition of AmpC activity by using cloxacillin-containing plates, PCR and

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Table 1
Limits of detection of SUPERCARBA medium for carbapenemase- and/or ESBL/AmpC-producing enterobacterial isolates as compared to those obtained with chromID OXA-48 and chromID CARBA media.

Strains	β-Lactamase content	MIC (μg/mL)			Lowest detection limit (CFU/plate)		
		IPMa	ETP	MEM	SUPER CARBA	chromID OXA-48	chromID CARBA
Ambler class D carbapenemases (n = 57)							
K. pneumoniae HPA	**OXA-48**[b] + TEM-1 + CTX-M-15 + OXA-1	1.5	>32	12	1×10^{2}	1×10^{1}	1×10^{4}[c]
K. pneumoniae VSG	**OXA-48** + TEM-1 + CTX-M-15 + OXA-1	0.75[d]	3	0.75	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae DUB	**OXA-48** + TEM-1 + CTX-M-15 + OXA-1 + CMY-2	3	>32	8	1×10^{2}	1×10^{2}	1×10^{2}
K. pneumoniae PLE	**OXA-48** + TEM-1	8	>32	6	1×10^{3}	1×10^{2}	1×10^{2}
K. pneumoniae KAY	**OXA-48** + TEM-1	0.5	0.75	0.25	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae TIK	**OXA-48** + OXA-1	0.75	2	0.38	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae VIL	**OXA-48** + CTXM-15 + TEM-1 + OXA-1	24	>32	16	1×10^{2}	1×10^{2}	1×10^{2}
K. pneumoniae ROB	**OXA-48** + CTXM-15 + TEM-1 + OXA-1	0.5	4	0.5	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae SCO	**OXA-48**	0.5	0.75	0.25	1×10^{1}	1×10^{2}	1×10^{5}
K. pneumoniae HT1	**OXA-48** + CTXM-15 + TEM-1 + SHV-1	2	>32	8	1×10^{1}	1×10^{2}	1×10^{2}
K. pneumoniae LOU	**OXA-48**	4	16	0.5	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae ROV	**OXA-48** + CTXM-15 + TEM-1 + OXA-1	0.5	3	0.38	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae 1963	**OXA-48** + CTXM-15 + TEM-1 + OXA-1	0.5	1	0.25	1×10^{2}	1×10^{2}	1×10^{5}
K. pneumoniae ELK	**OXA-48** + CTXM-15 + TEM-1 + OXA-1	0.5	3	0.38	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae AEL	**OXA-48** + CTX-M-15 + SHV-28 + OXA-1	0.5	6	0.38	1×10^{2}	1×10^{1}	1×10^{5}
K. pneumoniae SIC	**OXA-48** + CTX-M-15 + SHV-28 + TEM-1 + OXA-1	0.25	1	0.25	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae DUW	**OXA-48** + CTX-M-15 + TEM-1 + SHV-28 + OXA-1	32	32	32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae AMS	**OXA-48** + CTX-M-15 + TEM-1 + OXA-1	0.5	3	0.5	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae VER	**OXA-48** + CTX-M-15 + TEM-1	0.38	2	0.38	1×10^{2}	1×10^{1}	1×10^{5}
K. pneumoniae BAJ	**OXA-48** + CTX-M-15 + TEM-1 + SHV-28	0.5	1.5	0.38	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae BEN	**OXA-48** + CTX-M-15 + TEM-1 + SHV-28 + OXA-1	0.38	1	0.25	1×10^{2}	1×10^{1}	1×10^{5}
*K. pneumoniae*ZED	**OXA-48** + CTX-M-15 + TEM-1 + OXA-1	0.38	2	0.38	1×10^{2}	1×10^{2}	1×10^{5}
K. pneumoniae ALI	**OXA-48**	24	>32	16	1×10^{3}	1×10^{2}	1×10^{2}
K. pneumoniae SUL	**OXA-48** + CTXM-15 + TEM-1 + OXA-1 + CMY-2	1	3	3	1×10^{2}	1×10^{2}	1×10^{1}
K. pneumoniae KID	**OXA-48**	0.75	4	0.5	1×10^{2}	1×10^{1}	1×10^{5}
K. pneumoniae SAI	**OXA-48** + TEM-1	0.38	0.75	0.19	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae DIAR	**OXA-48** + CTXM-15 + TEM-1 + OXA-1	0.5	1	0.75	1×10^{1}	1×10^{2}	1×10^{5}
K. pneumoniae ORS	**OXA-48** + CTXM-15 + OXA-1	0.5	4	0.5	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae DOV	**OXA-48** + CTXM-15 + TEM-1 + OXA-1	3	1.5	0.25	1×10^{2}	1×10^{1}	1×10^{5}
K. pneumoniae LAS	**OXA-48** + CTXM-15 + TEM-1 + OXA-1	3	>32	8	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae BRE	**OXA-48** + CTXM-15 + TEM-1	0.5	1	0.38	1×10^{2}	1×10^{3}	1×10^{5}
K. pneumoniae 569400	**OXA-48** + CTXM-15 + TEM-1	0.38	0.75	0.19	1×10^{1}	1×10^{3}	1×10^{5}
K. pneumoniae ARA	**OXA-48** + CTXM-15 + TEM-1	0.5	1	0.38	1×10^{2}	1×10^{2}	1×10^{5}
E. coli 4	**OXA-48** + CTXM-14 + TEM-1	0.5	1.5	0.25	1×10^{1}	1×10^{1}	1×10^{5}
E. coli BOU	**OXA-48** + CTX-M-15	0.5	0.75	0.12	1×10^{2}	1×10^{2}	1×10^{5}
E. coli GOM	**OXA-48** + CTXM-15	0.5	1	0.19	1×10^{1}	1×10^{1}	1×10^{5}
E. coli ROV	**OXA-48** + TEM-1	0.5	0.75	0.25	1×10^{1}	1×10^{1}	1×10^{5}
E. coli ZAN	**OXA-48** + CTX-M-24 + TEM-1	0.38	8	0.75	1×10^{1}	1×10^{1}	1×10^{5}
E. coli LAL	**OXA-48** + CTX-M-15 + OXA-1	0.38	0.75	0.19	1×10^{2}	1×10^{5}	1×10^{5}
E. coli ESS	**OXA-48** + CTXM-15 + TEM-1	0.38	0.5	0.12	1×10^{1}	1×10^{2}	1×10^{5}
E. coli HANA	**OXA-48** + CTXM-15 + TEM-1	0.75	0.5	0.19	1×10^{1}	1×10^{1}	1×10^{5}
E. coli KID	**OXA-48** +TEM-1	0.38	0.5	0.12	1×10^{3}	1×10^{5}	1×10^{5}
E. coli MLI	**OXA-48** + VEB + TEM-1 + CMY-2	0.5	1	0.19	1×10^{1}	1×10^{1}	1×10^{5}
E. coli BAL	**OXA-48**	0.38	2	0.25	1×10^{1}	1×10^{2}	1×10^{5}
E. coli DOV	**OXA-48** + CTX-M-15 + TEM-1 + OXA-1	0.38	1.5	0.25	1×10^{1}	1×10^{1}	1×10^{5}
E. coli 11670	**OXA-48** + CTX-M-15 + TEM-1 + OXA-1	1.5	24	12	1×10^{1}	1×10^{1}	1×10^{5}
E. coli 11663	**OXA-48** + CTX-M-15 + TEM-1 + OXA-1	1.5	>32	8	1×10^{1}	1×10^{1}	1×10^{5}
E. cloacae BOU	**OXA-48** + CTX-M-15	1	4	1	1×10^{1}	1×10^{1}	1×10^{2}
K. oxytoca MAR21	**OXA-48** + CTX-M-15 + TEM-1	4	4	0.5	1×10^{1}	1×10^{1}	1×10^{5}
P. rettgerii	**OXA-48** + TEM-101	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{5}
S. marscecens	**OXA-48** + OXA-1	>32	>32	8	1×10^{1}	1×10^{1}	1×10^{1}
C. koseri ROU	**OXA-48**	0.38	2	0.38	1×10^{4}	1×10^{1}	1×10^{5}
C. koseri VER	**OXA-48** + TEM-1	0.75	2	0.38	1×10^{1}	1×10^{1}	1×10^{5}
K. pneumoniae KIRK	**OXA-162** + TEM-1 + SHV-11	4	8	1	1×10^{2}	1×10^{1}	1×10^{5}
K. pneumoniae HOL	**OXA-181** + CTX-M-15	1	4	1	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae 4799	**OXA-204** + CTX-M-14 + CMY-4 + OXA-1	0.5	2	2	1×10^{2}	1×10^{1}	1×10^{3}
K. pneumoniae DEL	**OXA-232** + TEM-1 + CTX-M-15 + OXA-1	3	>32	12	1×10^{2}	1×10^{5}	1×10^{1}
Ambler class A carbapenemases (KPC)(n = 10)							
K. pneumoniae KN633	**KPC-2** + TEM-1 + SHV-11 + OKPA + CTX-M-12	>32	>32	>32	1×10^{1}	1×10^{7}	1×10^{1}
K. pneumoniae A28006	**KPC-2** + TEM-1 + SHV-11 + CTX-M-2	16	24	32	1×10^{1}	1×10^{7}	1×10^{1}
K. pneumoniae A33504	**KPC-2** + TEM-1 + SHV-11 + CTX-M-2 + OXA-9	>32	>32	>32	1×10^{1}	1×10^{7}	1×10^{1}
K. pneumoniae 475	**KPC-2** + SHV-11 + CTX-M-15	16	>32	>32	1×10^{1}	1×10^{7}	1×10^{1}
K. pneumoniae MUS	**KPC-2** + TEM-1 + SHV-12	0.75	4	1.5	1×10^{1}	1×10^{7}	1×10^{1}
E. coli LIL	**KPC-2** + TEM-1 + OXA-9	2	1.5	1	1×10^{1}	1×10^{7}	1×10^{1}
E. coli PSP	**KPC-2** + TEM-1 + OXA-1	0.5	0.5	0.5	1×10^{6}	1×10^{7}	1×10^{1}
E. coli COL	**KPC-2** + TEM-1 + CTX-M-9	4	4	2	1×10^{1}	1×10^{7}	1×10^{1}
E. cloacae HMG	**KPC-2** + TEM-1	24	>32	16	1×10^{1}	1×10^{7}	1×10^{1}
C. freundii HPTU	**KPC-2** + TEM-1	8	1.5	3	1×10^{1}	1×10^{7}	1×10^{1}

(*continued on next page*)

Table 1 (continued)

Strains	β-Lactamase content	MIC (μg/mL)			Lowest detection limit (CFU/plate)		
		IPM[a]	ETP	MEM	SUPER CARBA	chromID OXA-48	chromID CARBA
Ambler class B carbapenemases (n = 10)							
K. pneumoniae 6759	**NDM-1** + CTX-M-15 + CMY-16 + OXA-1 + OXA-9 + OXA-10	12	>32	>32	1×10^1	$>1 \times 10^7$	1×10^1
K. pneumoniae KIE	**NDM-1** + SHV-38 + CMY-16+OXA-10	0.75	2	1	1×10^1	$>1 \times 10^7$	1×10^1
K. pneumoniae DIH	**VIM-19** + CTX-M-3 + TEM-1	8	16	4	1×10^1	$>1 \times 10^7$	1×10^1
K. pneumoniae KOW1	**VIM-4** + CTX-M-15 + TEM-1 + SHV-91	4	12	8	1×10^3	$>1 \times 10^7$	1×10^1
E. coli CHAN	**NDM-1** + TEM-1 +CTM-M-15 OXA-1	3	>32	4	1×10^1	$>1 \times 10^7$	1×10^1
E. coli FEK	**NDM-4** + CTX-M-15 + OXA-1	>32	>32	>32	1×10^1	$>1 \times 10^7$	1×10^1
E. coli KOW7	**VIM-4**	1	4	1	1×10^1	$>1 \times 10^7$	1×10^1
C. freundii MIG	**VIM-2** + TEM-1 + OXA-9 + OXA-10	1.5	4	0.5	1×10^3	$>1 \times 10^7$	1×10^4
S. typhimurium CAN	**NDM-1** + TEM-1 + CTX-M-15 +OXA-1 + OXA-9 + OXA-10	6	8	2	1×10^1	$>1 \times 10^7$	1×10^1
E. cloacae KAR	**VIM-1** + SHV-70	1	0.38	0.5	$>1 \times 10^7$	$>1 \times 10^7$	1×10^6
Non-carbapenemase producers with decreased susceptibility to carbapenems (n = 20)							
K. pneumoniae 648236[e]	SHV-2a	0.25	2	0.38	1×10^1	$>1 \times 10^7$	$>1 \times 10^7$
K. pneumoniae BER[e]	SHV-28 + TEM-1	1	4	1	1×10^1	$>1 \times 10^7$	1×10^6
K. pneumoniae MEK[e]	CTX-M-15 + SHV-11	1.5	>32	6	1×10^1	$>1 \times 10^7$	$>1 \times 10^7$
K. pneumoniae SIM[e]	CTX-M-15 + TEM-1 + SHV-1	8	>32	6	1×10^1	$>1 \times 10^7$	1×10^1
K. pneumoniae SHM[e]	CTX-M-15 +TEM-1 + SHV-11	3	>32	3	1×10^1	$>1 \times 10^7$	1×10^1
K. pneumoniae COO[e]	CTX-M-15 + SHV-28	8	>32	4	1×10^1	$>1 \times 10^7$	1×10^1
K. pneumoniae FOS[e]	CTX-M-15 + TEM-1 + SHV-11	6	>32	>32	1×10^1	$>1 \times 10^7$	1×10^1
K. pneumoniae BED[e]	CTX-M-15 + TEM-1 + SHV-11	1.5	>32	4	1×10^1	$>1 \times 10^7$	1×10^6
K. pneumoniae SHI[e]	CTX-M-15 + TEM-1 + SHV-11	0.25	1	1	1×10^4	$>1 \times 10^7$	$>1 \times 10^7$
K. pneumoniae LEG[e]	CTX-M-15 + TEM-1 + SHV-12	0.75	>32	3	1×10^4	$>1 \times 10^7$	1×10^4
K. pneumoniae ALE[e]	CTX-M-15 + SHV-1	1	>32	4	1×10^4	$>1 \times 10^7$	1×10^3
E. coli MAR[f]	Overexpressed AmpC	16	>32	2	1×10^1	$>1 \times 10^7$	1×10^1
E. cloacae BER[f]	Overexpressed AmpC	8	16	1.5	1×10^1	$>1 \times 10^7$	1×10^1
E. cloacae BLA[f]	Overexpressed AmpC	0.12	1	0.12	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. cloacae CON[f]	Overexpressed AmpC	0.25	4	0.25	1×10^5	$>1 \times 10^7$	$>1 \times 10^7$
E. cloacae AZA[f]	Overexpressed AmpC	0.12	1	0.12	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. cloacae COUP[f]	Overexpressed AmpC	4	32	2	1×10^1	$>1 \times 10^7$	1×10^7
E. cloacae JEN[f]	Overexpressed AmpC	1	1.5	0.12	1×10^4	$>1 \times 10^7$	$>1 \times 10^7$
E. cloacae RAY[f]	Overexpressed AmpC	1.5	1.5	0.09	1×10^5	$>1 \times 10^7$	1×10^6
C. freundii MAU[f]	Overexpressed AmpC + TEM-3	1	8	1	1×10^1	$>1 \times 10^7$	1×10^6
Non-carbapenemase producers being susceptible to carbapenems (n = 20) ESBLs							
K. pneumoniae 1022	SHV-2a + SHV-28	0.5	0.02	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
K. pneumoniae 10112	CTX-M-15 + TEM-1 + SHV-11	0.5	0.02	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
K. pneumoniae 1025	CTX-M-14 + TEM-1 + SHV-11	0.12	0.02	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. coli 1008	CTX-M-1 + TEM-1	0.19	0.02	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. coli 10121	CTX-M-2	0.19	0.02	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. coli 10122	CTX-M-1 + TEM-1	0.19	0.02	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. coli 1034	TEM-1 + SHV-38	0.19	0.01	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. coli 1048	TEM-1 + SHV-2a	0.19	0.01	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. aerogenes 1009	TEM-24	0.19	0.12	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. cloacae 1012	TEM-1 + SHV-12	0.19	0.01	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
Cephalosporinases							
E. cloacae 7746	(wild type)	0.38	0.09	0.03	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. cloacae 7725	(wild type)	0.19	0.01	0.01	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. aerogenes 0225	(wild type)	0.19	0.03	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
M. morganii 5902	(wild type)	1.5	0.01	0.06	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
C. freundii 7767	(wild type)	0.25	0.01	0.01	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. coli MET	ESAC [g]	0.12	0.12	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. coli GOU	DHA-1	0.12	0.01	0.01	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. coli BEL	ACC-1	0.19	0.05	0.02	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
P. mirabilis PMA	ACC-1	0.25	0.09	0.06	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$
E. coli SYD	CMY-2	0.12	0.25	0.03	$>1 \times 10^7$	$>1 \times 10^7$	$>1 \times 10^7$

MIC values of imipenem, ertapenem, and meropenem are provided for each strain.
[a]Abbreviations: IMP = imipenem; ETP = ertapenem; MP = meropenem.
[b]Boldened β-lactamase name correspond to carbapenemase.
[c]Underlined 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.
[d]Greyish values correspond to MIC values below the breakpoint of resistance for carbapenemase producers.
[e]Reduced susceptibility to ertapenem due to porin deficiency.
[f]Reduced susceptibility to ertapenem due to overexpressed AmpC.
[g]Chromosome-located expanded-spectrum AmpC.

sequencing of AmpC genes, biochemical Carba NP test) (Caroff et al., 2000; Nordmann et al., 2012c).

MICs of imipenem, ertapenem, and meropenem were determined by Etest and interpreted according to the updated Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2012) (Table 1). The lowest detection limit of any type of carbapenemase producers was determined by using chromID CARBA and the home-designed SUPERCARBA medium whereas that for the OXA-48 β-lactamase producers by using chromID OXA-48 (bioMérieux, La Balme-les-Grottes, France), chromID CARBA and SUPERCARBA (Table 1) (Nordmann et al., 2012b). Five inocula were tested ranging from 1×10^2 to 1×10^6 CFU/mL. Each inoculum was plated

Table 2
Sensitivity of SUPERCARBA, chromID OXA-48, chromID CARBA media, and combination of both chromID OXA-48 and chromID ARBA relatively to the average inoculum for detecting Ambler class D carbapenemase producers (n = 57).

Average inoculum (CFU/plate)	Sensitivity (%)			
	SUPERCARBA	chromID OXA-48	chromID CARBA	chromID OXA-48 + CARBA
10^1	61.4	66.7	10.5	70.2
10^2	93.0	91.2	21.1	93.0
10^3	98.2	94.7	22.8	96.5
10^4	100	94.7	24.6	96.5
10^5	100	96.5	28.1	98.2

on each screening medium (100 μL). Viable bacteria were counted after 24 h of culture at 37 °C. The performance of the three selective media was compared taking in account the bacterial inoculum (Table 2).

The lowest limit of detection of OXA-48-like producers ranged from 1×10^1 to 1×10^2 CFU/plate by using the SUPERCARBA and chromID OXA-48 media for most of the strains, whereas it was mainly $>1 \times 10^5$ CFU/plate by using the chromID CARBA (Table 1). Only three OXA-48-like strains (*E. coli* KID and *E. coli* LAL producing OXA-48 and *K. pneumoniae* DEL producing OXA-232) did not grow on the chromID OXA-48 medium (limit of detection $\geq 1 \times 10^5$ CFU/plate) (Table 1).

The SUPERCARBA medium has the highest sensitivity for detection of OXA-48 producers, ranging from 93–100%, respectively (for a low and a high inoculum) (Table 2). The chromID OXA-48 has also a high sensitivity, ranging from 91.2% to 96.5%, respectively, for a low (10^2 CFU/plate) and a high inoculum (10^5 CFU/plate). When using a very low inoculum (10^2 CFU/mL [10^1 CFU/plate]), chromID OXA-48 has a higher sensitivity than the SUPERCARBA medium being 66.7% versus 61.4 %. The chromID CARBA has a low sensitivity (below 30 %) for the detection of OXA-48-like producers whatever the inoculum was (Table 2). Specificity of chromID OXA-48 was the highest with 100 %, as compared to 52.5 % and 67.5 % for the SUPERCARBA and chromID CARBA media, respectively (Table 3).

An additional clinical study was performed with 120 clinical rectal swabs and 30 stool samples, containing no OXA-48-like producers, as assessed by molecular identification of the β-lactamase content of all isolates that grew on SUPERCARBA and/or chromID CARBA. Rectal swabs were resuspended in 1 mL of saline, and 1 g of stools was diluted in 10 mL of saline before plating (100 μL) on each screening medium. This study showed that the specificity of chromID OXA-48 was 100% using clinical samples, whereas it was 82% and 97% for SUPERCARBA and chromID CARBA, respectively (data not shown). The lower specificity of SUPERCARBA and chromID CARBA was mostly due to enterobacterial isolates with decreased susceptibility to carbapenems, and some with ertapenem MICs ranging from 0.5 to 1 mg/L (corresponding to susceptibility and intermediate susceptibility, according to the CLSI guidelines). A single *K. pneumoniae* isolate producing NDM-1 was detected on SUPERCARBA and chromID CARBA with an inoculum average of ca. 2×10^2 CFU/plate (data not shown). This isolate was not detected on chromID OXA-48, that is not intended to detect other carbapenemase than OXA-48-like producers.

The sensitivity of chromID CARBA was lower than that of SUPERCARBA for detection of OXA-48 producers (Table 2), but it was the same for detection of other classes of carbapenemase producers (90 %, Table 3). This result mirrored that of Vrioni et al. (2012) that show that chromID CARBA has an excellent ability to detect KPC and MBL producers (Vrioni et al., 2012).

In conclusion, the chromID OXA-48 was as sensitive for detection of OXA-48 producers as the SUPERCARBA medium is but with a higher specificity. The chromID CARBA medium, showing a weak sensitivity for detection of OXA-48 producers, is a powerful tool for detection of all other classes of CPE. As of both chromID media are complementary. The potential use of the novel chromID OXA-48 medium shall be for controlling an ongoing outbreak of OXA-48 producers or in combination with the chromID CARBA. The SUPERCARBA and the combination of chromID CARBA/chromID OXA-48 media offer the highest sensitivity for detecting any type of carbapenemases.

References

Adler A, Navon-Venezia S, Moran-Gilad J, Marcos E, Schwartz D, Carmeli Y. Laboratory and clinical evaluation of screening agar plates for detection of carbapenem-resistant *Enterobacteriaceae* from surveillance rectal swabs. J Clin Microbiol 2011;49:2239–42.

Caroff N, Espaze E, Gautreau D, Richet H, Reynaud A. Analysis of the effects of −42 and −32 *ampC* promoter mutations in clinical isolates of *Escherichia coli* hyperproducing ampC. J Antimicrob Chemother 2000;45:783–8.

Castanheira ML, Deshpande M, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1- and OXA-181-producing *Enterobacteriaceae* in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program, 2006–2007. Antimicrob Agents Chemother 2011;55:1274–8.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement (M100-S22). Wayne, PA: CLSI; 2012.

Landman D, Urban C, Bäcker M, Kelly P, Shah N, Babu E, Bratu S, Quale J. Susceptibility profiles, molecular epidemiology, and detection of KPC-producing *Escherichia coli* isolates from the New York City vicinity. J Clin Microbiol 2010;48:4604–7.

Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. Trends Microbiol 2011;19:588–95.

Nordmann P, Dortet L, Poirel L. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! Trends Mol Med 2012a;18:263–72.

Table 3
Sensitivity and specificity of SUPERCARBA, chromID OXA-48, chromID CARBA media, and combination of both chromID OXA-48 and chromID CARBA.

	SUPER CARBA	chromID OXA-48	chromID CARBA	chromID OXA-48 + CARBA
SN (%)[a]	96.1	70.1	40.3	94.8
SN class D carbapenemase	98.2	94.7	22.8	96.5
SN other classes of carbapenemases (A and B)[b]	90.0	0	90.0	90.0
SP (%)[b]	52.5	100	67.5	67.5
SP reduced susceptibility[c]	10.0	100	35.0	35.0
SP susceptible[c]	95.0	100	100	100

[a] 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-type; class B, of VIM and NDM-types; class D, of OXA-48-type.

[b] SP = specificity determined with cut off values set at 1×10^7 CFU/plate for non-carbapenemase producers susceptible or with reduced susceptibility to carbapenems.

Nordmann P, Girlich D, Poirel L. Detection of carbapenemase producers in *Enterobacteriaceae* using a novel screening medium. J Clin Microbiol 2012b;50:2761–6.
Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase producing Enterobacteriaceae. Emerg Infect Dis 2012c;18:1503–7.
Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother 2012;67:1597–606.
Vatopoulos A. High rates of metallo-β-lactamase-producing *Klebsiella pneumoniae* in Greece - a review of the current evidence. Euro Surveill 2008;13:1–6.
Vrioni G, Daniil I, Voulgari E, Ranellou K, Koumaki V, Ghirardi S, Kimouli M, Zambardi G, Tsakrisa A. Comparative evaluation of a prototype chromogenic medium (ChromID CARBA) for detecting carbapenemase-producing Enterobacteriaceae in surveillance rectal swabs. J Clin Microbiol 2012;50:1841–6.
Wilkinson KM, Winstanley TG, Lanyon C, Cummings SP, Raza MW, Perry JD. Comparison of four chromogenic culture media for carbapenemase-producing Enterobacteriaceae. J Clin Microbiol 2012;50:3102–4.