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CHROMagar mSuperCARBA and RAPIDEC® Carba NP test for detection of carbapenemase-producing Enterobacteriaceae

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ABSTRACT

A novel chromogenic medium CHROMagar mSuperCARBA was evaluated to detect carbapenem-resistant Gram-negatives. This medium is as sensitive and as specific as the SUPERCARBA medium for detecting KPC, MBL and OXA-48-type producers (100% and 100%, respectively) and is compatible with subsequent testing of carbapenemase activity using the RAPIDEC® CARBA NP.

Carbapenemase-producing Enterobacteriaceae (CPE) are increasingly reported worldwide (Pitout and Laupland, 2008). Mortality rates as high as 69% due to infections caused by these bacteria have been reported (Djahmi et al., 2014). Carbapenem resistance in Enterobacteriaceae has become worrisome since the spread of KPC-type carbapenemases among *Klebsiella pneumoniae* isolates in the 2000's (Yigit et al., 2001). Worldwide spread of carbapenemase producers has been facilitated by the mobilization of carbapenemase genes from mobile genetic elements (conjugative plasmids, integrons or transposons) among different enterobacterial species (Nordmann and Poirel, 2014). In this context, the rapid detection of these microorganisms is critical for preventing the development of nosocomial outbreaks.

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 ß-lactams except aztreonam, and the Ambler class D (OXA-48-like) carbapenemases, hydrolyzing carbapenems and broad-spectrum cephalosporins only weakly (Nordmann et al., 2011, 2012a).

Chromogenic and non-chromogenic screening methods for detecting CPE bacteria have been developed. Among chromogenic media, CHROMagar KPC (CHROMagar Ltd) is effective for detecting VIM and KPC carbapenemase producers (Kruse et al., 2013), but poorly detects OXA-48 producers (Girlich et al., 2013b; Nordmann et al., 2012b). *Brilliance* CRE (Oxoid, Thermofisher Scientific) is reported to more efficiently detect KPC- and MBL-producing Enterobacteriaceae (Girlich et al., 2013b), and the chromogenic medium chromID CARBA (bioMérieux) well detects CPE, except OXA-48 producers (Girlich et al., 2013a). Detection of OXA-48-like producers can be efficient using the chromID OXA-48 medium (bioMérieux) (Girlich et al., 2013a). The chromID CARBA SMART (bioMérieux) is a selective chromogenic bi-plate medium that selects OXA-48 and non-OXA-48 carbapenemase producers.

Recently, the SUPERCARBA medium has been developed and its performances have been compared to those of the chromID CARBA and chromID OXA-48 media (Girlich et al., 2013a), and to the *Brilliance* CRE and CHROMagar KPC media (Girlich et al., 2013b). Those studies showed that sensitivity of detection of CPE by SUPERCARBA medium is much higher than those obtained with *Brilliance* CRE and CHROMagar KPC (96.5% versus 76.3% and 43%, respectively), and the specificity (60.7%) is higher than that of the *Brillance* CRE medium (57.1%), although slightly lower than that of the CHROMagar KPC medium (67.8%). SUPERCARBA medium is as sensitive as the chromID OXA-48 medium for detection of OXA-48 producers, but with a lower specificity, and as sensitive as the chromID CARBA medium for detection of other classes of carbapenemase producers (90%). Overall, those results demonstrate that the SUPERCARBA medium may detect KPC, MBL and OXA-48 producers with high sensitivity. A pitfall of this medium is

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Table 1
Limits of detection of CHROMagar mSuperCARBA and SUPERCARBA media and Rapidec® Carba NP test for 117 carbapenemase- and/or ESBL/AmpC-producing enterobacterial isolates.

Strains	β-Lactamase content	MIC (mg/L)			Lowest detection limit (CFU/mL)[b]		
		IPM[a]	ETP	MEM	CHROMagar mSuperCARBA	SUPER CARBA	RAPIDEC® CARBA NP
Susceptible to carbapenems (n = 18)							
***E. cloacae* TH12**	VEB-1	1	0.25	0.12	$>10^7$	10^7	–
***E. coli* REG**	CTX-M-1	0.12	0.06	0.03	$>10^7$	$>10^7$	–
***E. coli* MAI**	CTX-M-14	0.12	0.03	0.03	$>10^7$	$>10^7$	–
E. coli	CTX-M-14	0.06	≤0.01	0.03	$>10^7$	$>10^7$	–
***E. coli* PAR**	CTX-M-14	0.25	0.06	0.03	$>10^7$	$>10^7$	–
***E. coli* DES**	CTX-M-15	0.12	0.06	≤0.01	$>10^7$	$>10^7$	–
***E. coli* UHR**	CTX-M-15	0.12	0.25	0.03	$>10^7$	10^7	–
***E. coli* MED**	CTX-M-15	0.5	0.06	0.06	$>10^7$	$>10^7$	–
***E. coli* 764**	CTX-M-15	0.25	0.06	0.03	$>10^7$	10^6	–
***E. coli* ABD**	CTX-M-15	0.12	0.12	0.03	$>10^7$	10^6	–
***E. coli* CAL**	CTX-M-15	0.5	0.12	0.06	$>10^7$	10^7	–
E. cloacae	CTX-M-15	1	0.25	0.06	$>10^7$	10^7	–
***E. coli* LAG**	CTX-M-27	0.12	≤0.01	0.03	$>10^7$	$>10^7$	–
***E. coli* THAI3**	VEB-1	0.12	0.01	0.03	$>10^7$	$>10^7$	–
***E. coli* LOU**	ACC-1	0.25	0.03	0.03	$>10^7$	$>10^7$	–
***K. pneumoniae* MOR-1**	DHA-2	0.5	1	0.12	10^7	$>10^7$	–
***P. mirabilis* BEL**	ACC-1	4	0.03	0.12	$>10^7$	$>10^7$	–
***E. coli* MET**	Chromosome-encoded extended-spectrum cephalosporinase	0.25	0.25	0.03	$>10^7$	$>10^7$	–
Reduced susceptibility to carbapenems (ESBL/overexpressed AmpC/porin deficiency) (n = 13)							
***K. pneumoniae* MEK**[d]	CTX-M-15 + SHV-11	1.5	>32	6	10^1	10^1	–
***K. pneumoniae* FOS**[d]	CTX-M-15 + TEM-1 + SHV-11	6	>32	>32	10^1	10^2	–
***K. pneumoniae* BER**[d]	TEM-1 + SHV-28	1	4	1	10^1	10^2	–
***K. pneumoniae* ALE**[d]	CTX-M-15 + SHV-1	1	>32	4	10^1	10^5	–
***K. pneumoniae* SIM**	CTX-M-15 + TEM-1 + SHV-1	8	>32	6	10^1	10^1	–
***K. pneumoniae* SHM**	CTX-M-15 + TEM-1 + SHV-11	3	>32	3	10^2	10^1	–
***K. pneumoniae* COO**	CTX-M-15 + SHV-28	8	>32	4	10^1	10^1	–
***K. pneumoniae* 648,236**	SHV2a	0.25	2	0.38	10^1	10^2	–
***K. pneumoniae* BED**	CTX-M-15 + TEM-1 + SHV-11	2	>32	8	10^1	10^1	–
K. pneumoniae	CTX-M-15 + TEM-1 + SHV-11	2	>32	8	10^1	10^1	–
K. pneumoniae	CTX-M-15 + TEM-1 + SHV-1	0.38	24	1.5	10^2	10^1	–
***E. coli* MAR**[e]	Overexpressed AmpC	16	>32	2	10^1	5×10^3	–
***E. coli* SW**	CTX-M-15	1	24	4	10^1	10^1	–
KPC producers (n = 17)							
***K. pneumoniae* SAG**	**KPC-2**[c] + OXA-9 + TEM-1	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* THO**	**KPC-2** + OXA-9 + TEM-1	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* TIF**	**KPC-2** + OXA-9 + TEM-1	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* LIE**	**KPC-2** + OXA-9 + TEM-1	>32	>32	>32	10^1	5×10^1	+
***K. pneumoniae* 588**	**KPC-2** + OXA-9 + SHV-11 + TEM-1	24	32	16	10^1	10^1	+
***K. pneumoniae* A33504**	**KPC-2** + CTX-M-2 + SHV-11 + OXA-9 + TEM-1	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* 475**	**KPC-2** + CTX-M-15 + SHV-11	16	>32	>32	10^1	10^1	+
***E. coli* COL**	**KPC-2** + CTX-M-9 + TEM-1	4	4	2	10^1	10^1	+
***E. cloacae* HPT2**	**KPC-2**	1	1.5	0.75	10^1	10^1	+
***E. cloacae* CFVL**	**KPC-2** + TEM-3	4	2	1	10^1	10^1	+
***K. pneumoniae* SUZ**	**KPC-3**	32	>32	32	10^1	10^1	+
***K. pneumoniae* JUL**	**KPC-3**	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* ELB**	**KPC-3**	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* TER**	**KPC-3**	32	>32	>32	10^2	10^2	+
***K. pneumoniae* CHRIS**	**KPC-3** + TEM-1 + SHV-11 + OXA-9	32	>32	32	10^1	10^1	+
***K. pneumoniae* GRE**	**KPC-3** + TEM-1 + SHV-11 + OXA-9	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* BEN**	**KPC-3** + TEM-1 + SHV-11 + OXA-9	32	>32	32	10^1	10^2	+
Metallo-ß-lactamases (n = 33)							
***K. pneumoniae* 10MA**	**NDM-1** + CTX-M-15 + TEM-1 + SHV-11 + SHV-28 + OXA-1 + OXA-9	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* IND**	**NDM-1** + CTX-M-15 + TEM-1 + SHV-28 + CMY-6 + OXA-1 + OXA-9	1	8	4	10^1	10^1	+
***E. coli* RIC**	**NDM-1** + TEM-1 + CMY-16 + OXA-1 + OXA-10	1	3	1	10^1	10^1	+
***E. coli* GUE**	**NDM-1** + TEM-1 + OXA-1	3	3	2	10^1	10^1	+
***E. coli* ALL**	**NDM-1** + CTX-M-15 + TEM-1 OXA-1 + OXA-2	4	>32	8	10^1	10^1	+
***E. coli* IR5**	**NDM-1** + CTX-M-15 + TEM-1	16	>32	16	10^1	10^1	+
***E. coli* FEK**	**NDM-4** + OXA-1 + CTX-M-15	32	>32	>32	10^1	10^1	+
***E. coli* I5**	**NDM-4** + CMY-6 + CTX-M-15	32	>32	>32	10^1	10^1	+
***E. coli* 405**	**NDM-5** + TEM-1 + CTX-M-15	32	>32	>32	10^1	10^2	+
***E. coli* GAL**	**NDM-6** + OXA-1 + CTX-M-15	32	>32	>32	10^1	10^1	+
***E. coli* THA**	**NDM-7**	>32	>32	>32	10^1	10^1	+
***E. coli* REI**	**NDM-7**	32	>32	>32	10^2	10^2	+
***K. pneumoniae* 0404020**	**VIM-1** + SHV-5	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* 0404024**	**VIM-1**	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* 0511135**	**VIM-1** + SHV-12	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* 1,008,055**	**VIM-1** + SHV-5	4	2	2	10^1	10^1	+
***K. pneumoniae* 1,108,007**	**VIM-1** + SHV-1	>32	4	4	10^1	10^1	+
***K. pneumoniae* 1,108,009**	**VIM-1** + SHV-12	8	>32	2	10^2	10^1	+
***K. pneumoniae* 1,108,015**	**VIM-1** + SHV-12	2	4	1	10^2	10^1	+

Table 1 *(continued)*

Strains	β-Lactamase content	MIC (mg/L)			Lowest detection limit (CFU/mL)[b]		
		IPM[a]	ETP	MEM	CHROMagar mSuperCARBA	SUPER CARBA	RAPIDEC® CARBA NP
***K. pneumoniae* 1,108,016**	**VIM-1** + SHV-5	8	>32	2	10^2	10^1	+
***E. coli* 0404018**	**VIM-1** + CMY-13	3	1.5	1	10^1	5×10^1	+
***E. cloacae* 1,008,029**	**VIM-1** + CTX-M-3	>32	>32	>32	10^1	2×10^1	+
***E. cloacae* 1,008,073**	**VIM-1**	4	>32	2	10^1	2×10^1	+
***S. marcescens* 1,008,091**	**VIM-1** + CTX-M-15	>32	>32	>32	10^1	10^1	+
***E. coli* KOW7**	**VIM-4**	16	16	8	10^1	10^1	+
***K. pneumoniae* 0709127**	**IMP-1** + TEM-1	0.5	4	1	10^1	10^1	+
***K. pneumoniae* 0709124**	**IMP-1** + TEM-15	8	3	2	10^1	10^1	+
***E. coli* 1,108,013**	**IMP-1** + TEM-1	0.5	4	1	10^1	10^1	+
***E. cloacae* 1,008,187**	**IMP-1**	8	>32	4	10^1	10^1	+
***S. marcescens* 1,008,175**	**IMP-1**	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* TWA**	**IMP-8**	1	1	0.5	10^1	10^1	+
***E. cloacae* TWA**	**IMP-8**	1.5	1	1	10^1	10^1	+
***S. marcescens* IBM19**	**IMP-11**	2	8	4	10^2	10^1	+
Carbapenemase of the OXA-48-type (n = 36)							
***K. pneumoniae* RAM**	**OXA-48**	1	4	1	10^1	10^1	+
***K. pneumoniae* LIB**	**OXA-48**	16	16	16	10^1	10^1	+
***K. pneumoniae* TIK**	**OXA-48**	0.75	2	0.38	10^1	10^1	+
***Enterobacter* spp. TUR9**	**OXA-48**	0.38	3	0.38	10^1	10^1	+
***E. cloacae* TUR10**	**OXA-48**	0.38	4	0.38	10^1	10^1	+
***K. pneumoniae* T12**	**OXA-48**	1.5	4	0.75	10^1	10^1	+
***K. pneumoniae* OM14**	**OXA-48** + TEM1	0.5	1	0.38	10^1	10^1	+
***K. pneumoniae* BOU**	**OXA-48** + CTX-M-15	0.38	0.5	0.25	10^2	10^1	+
***K. pneumoniae* EGY**	**OXA-48** + CTX-M-15	2	3	2	10^1	10^1	+
***K. pneumoniae* ROU**	**OXA-48** + CTX-M-15	0.5	1.5	0.25	10^1	10^1	+
***K. pneumoniae* BAJ**	**OXA-48** + CTX-M-15 + SHV-28 + TEM-1	0.5	1.5	0.38	10^1	10^1	+
***K. pneumoniae* BEN**	**OXA-48** + CTX-M-15 + SHV-28 + TEM-1	0.38	1	0.25	10^1	10^1	+
***K. pneumoniae* DUW**	**OXA-48** + CTX-M-15 + SHV-28 + TEM-1	32	32	32	10^1	10^1	+
***K. pneumoniae* SIC**	**OXA-48** + CTX-M-15 + SHV28	0.25	1	0.25	10^1	10^1	+
***K. pneumoniae* AMS**	**OXA-48** + CTX-M-15 + TEM-1 + OXA-1	0.5	2	0.38	10^1	10^1	+
***K. pneumoniae* ELK**	**OXA-48** + CTX-M-15 + TEM-1 + SHV-11	0.5	3	0.38	10^1	10^1	+
***K. pneumoniae* VSG**	**OXA-48** + CTX-M-15 + OXA-1 + TEM-1	0.75	3	0.75	10^1	10^1	+
***K. pneumoniae* HPA**	**OXA-48** + CTX-M-15 + OXA-1 + TEM-1	1.5	>32	12	10^1	10^1	+
***K. pneumoniae* OM11**	**OXA-48** + CTX-M-14 + TEM-1	0.5	0.75	0.25	10^1	10^1	+
***E. coli* BOU**	**OXA-48** + CTX-M-15	0.5	0.75	0.12	10^1	10^1	+
***E. coli* BON**	**OXA-48** + CTX-M-24 + TEM-1	0.38	0.5	0.19	10^1	10^2	+
***E. cloacae* TUR**	**OXA-48** + SHV-5	0.5	0.5	0.5	10^1	10^1	+
***K. pneumoniae* Af18**	**OXA-181**	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* Af54**	**OXA 181**	>32	>32	32	10^1	10^1	+
***K. pneumoniae* Af56**	**OXA-181**	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* Af39**	**OXA-181**	1	>32	12	10^1	10^1	+
***K. pneumoniae* Af53**	**OXA-181**	3	>32	4	10^1	10^1	+
***K. pneumoniae* DEL**	**OXA-181** + SHV11 + CTX-M-15 + TEM-1	>32	>32	>32	10^1	10^1	+
***K. pneumoniae* HOL**	**OXA-181** + CTX-M-15	8	>32	32	10^1	10^1	−
***E. coli* LIEU**	**OXA-181** + CTX-M-15	1	1	0.25	10^2	10^2	+
***K. pneumoniae* 479**	**OXA-204** + CMY-4	8	16	8	10^1	10^1	+
***E. coli* GRA**	**OXA-204** + CMY-2 + CTX-M-15 + OXA-1	2	1	0.25	10^2	10^1	+
***E. coli* DUP**	**OXA-204** + CMY-4 + CTX-M-15 + OXA-1	2	1	0.25	10^1	10^2	+
***E. coli* BAR**	**OXA-204** + CMY-4 + CTX-M-15	2	2	0.5	10^2	10^2	+
***K. pneumoniae* DEL**	**OXA-232** + SHV-1 + TEM-1 + CTX-M-15 + OXA-1	8	>32	32	10^2	10^2	−
***K. pneumoniae* RAN**	**OXA-232** + SHV-1 + TEM-1 + CTX-M-15 + OXA-1 + NDM-1	>32	>32	>32	10^1	10^1	+

[a] IPM = Imipenem; ETP = ertapenem; MEM = meropenem.
[b] Underlined colony-forming unit counts are considered as negative results (cut-off values set at ≥1 × 10^3 CFU/plate).
[c] Boldened β-lactamase names correspond to carbapenemase.
[d] Reduced susceptibility to ertapenem due to porin deficiency.
[e] Reduced susceptibility to ertapenem due to overexpressed AmpC coupled or not to porin deficiency.

that it does not include chromogenic molecules indentifying enterobacterial species.

Therefore, the chromogenic screening medium CHROMagar mSuperCARBA (CHROMagar company) was developed for the detection and presumed identification of carbapenemase-producing Enterobacteriaceae. The aim of this study was to compare the performance of the CHROMagar mSuperCARBA medium with the SUPERCARBA medium and to analyze the performances of the subsequent use of the RAPIDEC® Carba NP test for detection of carbapenemase activity.

A total of 117 clinical strains of Enterobacteriaceae were used. They had been previously characterized at the molecular level for their β-lactamase content. These strains were isolated from various clinical samples (blood cultures, urine, etc...) and represented the most frequent enterobacterial species producing carbapenemases worldwide. This collection included 18 susceptible-carbapenem isolates, 13 strains with reduced susceptibility to carbapenems but non-carbapenemase producers (ESBL, overexpressed AmpC and/or porin deficiency), and a series of 86 carbapenemase producers including 36 OXA-48-type producers, 17 KPC producers, 12 NDM producers, 13 VIM producers, and 8 IMP producers. Minimal inhibitory concentrations (MICs) of imipenem, ertapenem and meropenem were determined by Etest and MIC values were interpreted according to the 2014 Clinical and Laboratory Standard Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2014).

The SUPERCARBA plates were prepared as described previously (Nordmann et al., 2012b) and the new CHROMagar mSuperCARBA plates were prepared following the manufacturer's instructions. The inoculum of 0.5 McFarland corresponds to approximately 10^8 CFU/mL, and 100 μL of serial 10-fold dilutions were plated on the selective media SUPERCARBA NP and CHROMagar mSuperCARBA. Viable bacteria were counted from growth on Lisogenic Broth (LB) (Sigma) plates after 24 hours. Sensitivity and specificity were calculated for every medium and a limit value of 1×10^3 CFU/plate was considered as negative result, as previously described (Girlich et al., 2013a, b).

CHROMagar mSuperCARBA medium showed 100% sensitivity and 100% specificity for KPC, NDM, VIM, IMP and more interestingly OXA-48-type producers were better selected compared to SUPERCARBA medium (Table 1). This is noteworthy considering that the SUPERCARBA medium has the highest sensitivity for detection of OXA-48 producers either with low or high inoculums (93–100%, respectively) (Girlich et al., 2013a, b). Moreover, 100% of sensitivity and specificity was encountered for bacteria with reduced susceptibility. Noticeably, 2 additional isolates with reduced susceptibility to carbapenems were selected by the CHROMagar mSuperCARBA medium as compared to the SUPERCARBA medium. The limit of detection of those strains on the SUPERCARBA medium was indeed higher than the 10^3 CFU/plate cut-off value with SUPERCARBA medium, but this problem was not observed with the novel CHROMagar mSuperCARBA medium. On the other hand, chromogenic reactions of this novel medium produced the expected species identification (data not shown).

In addition, we evaluated the incompatibility of using first bacteria grown on CHROMagar mSuperCARBA medium and then the RAPIDEC® CARBA NP test. A total of 99 strains grown on this chromogenic medium (10 μL loop of bacterial colonies) were analyzed according to the manufacturer's instructions. Clear positive results for KPC, NDM, VIM and IMP producers were observed, while there was some variable degree of color change among the OXA-48-like producers (Table 1). Two OXA-48-like (OXA-181 and OXA-232) producers that were negative for RAPIDEC® CARBA NP test were negative even when bacteria were grown in LB plates showing that there was no chromogenic interference.

In conclusion, our results indicate that using first the novel chromogenic CHROMagar mSuperCARBA medium to select for carbapenem-resistant isolates and then the RAPIDEC® CARBA NP test offers a valuable option for screening carbapenemase producers.

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Conflicts of interest

An international patent form for the SUPERCARBA medium (that included the further development such of the mSupercarba medium) has been filled on behalf of INSERM Transfert (Paris, France).

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