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Detection of Carbapenemase Producers in *Enterobacteriaceae* by Use of a Novel Screening Medium

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A Drigalski agar-based culture medium containing an ertapenem, cloxacillin, and zinc sulfate (Supercarba medium) was tested for screening carbapenemase-producing members of the family *Enterobacteriaceae*. OXA-48 ($n = 44$), NDM ($n = 25$), VIM or IMP ($n = 27$), and KPC producers ($n = 18$) were detected with a low detection limit. Its overall sensitivity (95.6%) was higher than those of the currently available ChromID ESBL (bioMérieux) and CHROMagar KPC (CHROMagar) screening media. The Supercarba medium provides a significant improvement for detection of the most common types of carbapenemase producers.

A variety of carbapenemases are increasingly reported in members of the family *Enterobacteriaceae* worldwide. Carbapenemase producers are becoming a source of therapeutic failures in both hospital- and community-acquired infections. The detection of infected patients and carriers with multidrug-resistant isolates is therefore becoming a major issue, and it is a major health issue to prevent the spread of these isolates. The clinically significant carbapenemases in *Enterobacteriaceae* belong to several Ambler classes of β-lactamases that differ by chemical structures and biochemical properties (1). They are mostly of the Ambler class A (KPC) that hydrolyze all β-lactams, of the zinc-dependent Ambler class B (NDM, VIM, and IMP) that hydrolyze all β-lactams except aztreonam, and of the Ambler class D (OXA-48-like) that hydrolyze carbapenems and weakly hydrolyze (or do not hydrolyze) broad-spectrum cephalosporins (2, 5, 6, 8, 13, 15–17, 20–22). The level of resistance to carbapenems provided by those carbapenemase producers may vary significantly, making their detection difficult when based only on high-level carbapenem resistance (3, 4, 11, 12). A medium initially designed to screen for extended-spectrum β-lactamase (ESBL) producers that contains cefpodoxime (ChromID ESBL; bioMérieux, La Balme-les-Grottes, France) and a carbapenem-containing medium (CHROMagar KPC; CHROMagar Company, Paris, France) (11, 23, 24) were evaluated for screening carbapenemase producers. Both media contained chromogenic molecules that may contribute to the recognition of enterobacterial species. The ChromID ESBL medium has good sensitivity; its main disadvantage is its lack of detection of OXA-48-like producers that are susceptible to cefpodoxime in the absence of coproduction of an ESBL (3). In addition, this medium lacks specificity, since the widespread ESBL producers may be coselected on that medium. The CHROMagar KPC medium detects carbapenemase producers only if they are resistant to high levels of carbapenems. Therefore, its main disadvantage remains its lack of sensitivity, since it does not detect carbapenemase producers with a low level of resistance to carbapenems (3, 16). This is the case for many KPC-, IMP-, VIM-, NDM-, and OXA-48-producing *Escherichia coli* and *Klebsiella pneumoniae*.

Taking into account the current importance of detecting carbapenemase producers with accuracy, we have designed a novel screening medium called Supercarba medium. The rationale for the design of this medium was that it should be able to detect carbapenemase producers with low-level resistance to carbapenems and be as selective as possible by inhibiting the growth of carbapenem-resistant but non-carbapenemase-producing isolates.

Different concentrations of several carbapenem molecules were tested, and finally, ertapenem was added to Drigalski agar medium at a concentration of 0.25 μg/ml. $ZnSO_4$ (70 μg/ml) was added to improve expression of metallo-β-lactamases (MBLs) by MBL producers (12). Cloxacillin (250 μg/ml), which is a cephalosporinase (AmpC-type β-lactamase) inhibitor, was used to prevent growth of isolates expressing high levels of cephalosporinases, such as *Enterobacter cloacae*, *Enterobacter aerogenes*, *Morganella morgannii*, and *Serratia marcescens*. These isolates are clinically significant sources of carbapenem resistance associated with an outer membrane permeability defect (9, 14).

A total of 114 carbapenemase-producing isolates belonging to various enterobacterial species of worldwide origin were included in the study, all having a β-lactamase content characterized at the molecular level (Table 1). The strains were as follows: KPC producers ($n = 18$), VIM producers ($n = 12$), IMP producers ($n = 15$), NDM-1 producers ($n = 25$), together with OXA-48- ($n = 41$) and OXA-181 producers ($n = 3$). Seventy-five of those isolates coexpressed an ESBL (Table 1). Strains that did not express any carbapenemase were used as controls, consisting of isolates showing reduced susceptibility to ertapenem due to an overexpressed AmpC ($n = 10$), or to an ESBL ($n = 12$), and/or porin deficiency. Wild-type ertapenem-susceptible isolates, restricted-spectrum β-lactamase producers, ESBL producers, and high-level AmpC producers were also included as controls ($n = 40$) (Table 1). Using an inoculum of ~2×10^7 CFU/ml (range, 1.5×10^7 to 3.5×10^8 CFU/ml), serial 10-fold dilutions of the isolates were made in normal saline, and 100-μl portions were plated onto the Supercarba medium and compared to results obtained using CHROMagar KPC and ChromID ESBL media. Viable bacteria

Received 22 November 2011 Returned for modification 21 December 2011 Accepted 15 February 2012

Published ahead of print 22 February 2012

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doi:10.1128/JCM.06477-11

TABLE 1 MICs and limits of detection of Supercarba, ChromID ESBL, and CHROMagar KPC media[a]

Strain	β-Lactamase content[b]	MIC (μg/ml) of antibiotic[c]			Lowest detection limit (CFU/ml) for the following medium[d]:		
		IPM	ETP	MEM	Supercarba	ChromID ESBL	CHROMagar KPC
Ambler class A carbapenemase (KPC)-producing strains							
K. pneumoniae 2303	**KPC-2** + SHV-11	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae LIE	**KPC-2** + TEM-1 + OXA-9	>32	>32	>32	5×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae GES	**KPC-2** + TEM-1 + SHV-11	6	12	1.5	1×10^{1}	1×10^{1}	1×10^{3}
K. pneumoniae 588	**KPC-2** + TEM-1 + SHV-11 + OXA-9	24	32	16	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae YC	**KPC-2** + TEM-1 + SHV-11 + SHV-12 + OXA-9	4	24	2	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae A28006	**KPC-2** + TEM-1 + CTX-M-2 + SHV-11	16	24	32	2×10^{1}	1×10^{1}	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^{1}	1×10^{1}
K. pneumoniae MUS	**KPC-2** + TEM-1 + SHV-11 + SHV-12	0.75	4	1.5	1×10^{1}	1×10^{1}	1×10^{3}
K. pneumoniae KAM	**KPC-3** + TEM-1 + SHV-11	8	12	2	1×10^{1}	1×10^{1}	5×10^{3}
E. coli PSP	**KPC-2** + TEM-1 + OXA-1	0.5	0.5	0.5	1×10^{2}	1×10^{1}	1×10^{4}
E. coli DIN	**KPC-2** + TEM-1 + OXA-1	1	>32	0.5	1×10^{1}	1×10^{1}	1×10^{1}
E. coli COL	**KPC-2** + TEM-1 + CTX-M-9	4	4	2	1×10^{1}	1×10^{1}	1×10^{3}
E. coli LIL	**KPC-2** + TEM-1 + OXA-9	2	1.5	1	1×10^{1}	1×10^{1}	1×10^{1}
E. cloacae HMG	**KPC-2** + TEM-1	24	>32	16	1×10^{2}	1×10^{1}	1×10^{1}
E. cloacae CFVL	**KPC-2** + TEM-3	4	2	1	1×10^{1}	1×10^{1}	5×10^{5}
E. cloacae HPTU	**KPC-2** + TEM-1 + SHV-11	2	4	1.5	1×10^{1}	1×10^{1}	1×10^{1}
S. marcescens D6403	**KPC-2** + TEM-1	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
S. marcescens C7052	**KPC-2** + TEM-1 + SHV-12	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
Ambler class B carbapenemase-producing strains							
K. pneumoniae OMA419	**NDM-1** + OXA-1	1.5	6	2	1×10^{1}	1×10^{1}	1×10^{2}
K. pneumoniae KI2	**NDM-1** + CTX-M-15 + OXA-1	1	8	4	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae UK	**NDM-1** + CTX-M-15 + CMY-4 + OXA-1	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae 6642 GEN	**NDM-1** + CTX-M-15 + OXA-1 + OXA-10	1	16	3	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae 6759 GEN	**NDM-1** + CTX-M-15 + CMY-16 + OXA-1 + OXA-9 + OXA-10	12	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae OMA601	**NDM-1** + CTX-M-15 + OXA-1 + OXA-9	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae 7AFR	**NDM-1** + TEM-1 + CTX-M-15 + CMY-6 + OXA-1	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae OM2	**NDM-1** + TEM-1+ CTX-M-3 + SHV-11 + OXA-1	0.75	8	1.5	1×10^{1}	1×10^{1}	3×10^{4}
K. pneumoniae OM4	**NDM-1** + TEM-1 + CTX-M-15 + SHV-12 + OXA-9	4	>32	16	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae OM8	**NDM-1** + TEM-1 + CTX-M-15 + SHV-11 + OXA-1	2	>32	4	2×10^{1}	1×10^{1}	1×10^{2}
K. pneumoniae OM13	**NDM-1**+ TEM-1 + CTX-M-15 + SHV-28 + OXA-1 + OXA-9	3	4	2	1×10^{1}	1×10^{1}	3×10^{4}
K. pneumoniae OM15	**NDM-1** + CTX-M-15 + SHV-130 + OXA-1	1.5	12	3	1×10^{1}	1×10^{1}	3×10^{5}
K. pneumoniae OM16	**NDM-1** + CTX-M-15 + OXA-1 + **OXA-181**	8	>32	16	3×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae OM19	**NDM-1** + CTX-M-15 + SHV-12 + OXA-1	4	24	8	1×10^{1}	1×10^{1}	4×10^{2}
K. pneumoniae KIE	**NDM-1** + SHV-38 + CMY-16 + OXA-10	0.75	2	1	1×10^{1}	1×10^{1}	1×10^{4}
E. coli GUE	**NDM-1** + TEM-1 + OXA-1	3	3	2	1×10^{1}	1×10^{1}	1×10^{5}
E. coli AUS	**NDM-1** + TEM-1 + CTX-M-15	6	32	16	1×10^{1}	1×10^{1}	1×10^{1}
E. coli IR5	**NDM-1** + TEM-1 + CTX-M-15	16	>32	16	1×10^{1}	1×10^{1}	1×10^{1}
E. coli GEN	**NDM-1** + TEM-1 + CMY-30 + OXA-1	8	>32	12	1×10^{1}	1×10^{1}	1×10^{1}
E. coli RIC	**NDM-1** + CMY-16 + OXA-1 + OXA-10	1	3	1	1×10^{1}	1×10^{1}	1×10^{5}
E. coli ALL	**NDM-1** + TEM-1 + CTX-M-15 + OXA-1 + OXA-2	4	>32	8	1×10^{1}	1×10^{1}	1×10^{1}
E. coli OM20	**NDM-1**+ TEM-1 + CTX-M-15	2	>32	8	1×10^{1}	1×10^{1}	1×10^{1}
E. cloacae IR38	**NDM-1** + CTX-M-15	2	16	2	1×10^{1}	3×10^{2}	4×10^{4}
P. stuartii PS1	**NDM-1** + CMY-6 + OXA-1	12	0.38	1.5	1×10^{7}	1×10^{3}	1×10^{7}
C. freundii STE	**NDM-1** + TEM-1 + CTX-M-15 + **VIM-4** + OXA-1 + OXA-9 + OXA-10 + **OXA-181**	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae 0404024	**VIM-1**	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae 0511135	**VIM-1** + SHV-12	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae 0404020	**VIM-1** + SHV-5	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae ENN	**VIM-1** + SHV-5	0.5	1.5	0.38	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae MAD	**VIM-1** + CTX-M-3	1	0.5	1	1×10^{1}	3×10^{1}	2×10^{4}
E. coli DIH	**VIM-19**	8	16	4	1×10^{1}	1×10^{1}	1×10^{1}
E. coli 0404018	**VIM-1** + CMY-6	3	1.5	1	5×10^{1}	1×10^{1}	$>1 \times 10^{8}$
E. coli 1008077	**VIM-1** + TEM-1 + CTX-M-15	>32	4	4	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
E. coli MAD	**VIM-1** + CTX-M-3	1.5	0.38	0.5	1×10^{5}	1×10^{1}	2×10^{5}
E. cloacae KAR	**VIM-1** + SHV-70	1	0.38	0.5	1×10^{6}	1×10^{1}	$>1 \times 10^{8}$
E. cloacae 1008029	**VIM-1** + CTX-M-3	>32	>32	>32	2×10^{1}	1×10^{1}	1×10^{1}
S. marcescens 1008091	**VIM-1** + CTX-M-15	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae TUR	**IMP-1**	1	2	8	1×10^{6}	2×10^{1}	1×10^{1}
K. pneumoniae 0709121	**IMP-1**	1.5	3	1	1×10^{1}	1×10^{1}	1×10^{3}
K. pneumoniae 0709124	**IMP-1** + TEM-15	8	3	2	1×10^{1}	1×10^{1}	1×10^{4}
K. pneumoniae 0709125	**IMP-1** + TEM-1 + SHV-12	1.5	4	2	1×10^{1}	1×10^{1}	1×10^{3}
K. pneumoniae 0709127	**IMP-1** + TEM-1	0.5	4	1	1×10^{1}	1×10^{1}	1×10^{4}

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TABLE 1 (Continued)

Strain	β-Lactamase content[b]	MIC (μg/ml) of antibiotic[c]			Lowest detection limit (CFU/ml) for the following medium[d]:		
		IPM	ETP	MEM	Supercarba	ChromID ESBL	CHROMagar KPC
K. pneumoniae TWA	**IMP-8**	1	1	0.5	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae TAW	**IMP-8** + SHV-12	0.5	0.5	0.5	4×10^{2}	1×10^{1}	$>1 \times 10^{8}$
E. coli JAP	**IMP-1**	0.5	3	0.5	1×10^{4}	1×10^{1}	2×10^{5}
E. coli TWA	**IMP-8** + SHV-12	6	8	3	1×10^{1}	1×10^{1}	1×10^{1}
E. coli 1108013	**IMP-1** + TEM-1	0.5	4	1	1×10^{1}	1×10^{1}	1×10^{6}
E. cloacae TWA	**IMP-8**	1.5	1	1	1×10^{1}	1×10^{1}	1×10^{2}
E. cloacae TAW	**IMP-8** + SHV-12	0.75	0.5	0.5	1×10^{2}	1×10^{1}	$>1 \times 10^{8}$
E. cloacae 1008079	**IMP-1**	8	>32	>32	1×10^{1}	1×10^{2}	1×10^{7}
E. cloacae 1008187	**IMP-1** + CTX-M-15	8	>32	4	1×10^{1}	1×10^{1}	1×10^{4}
S. marcescens 0911033	**IMP-1**	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
Ambler class D carbapenemase-producing strains							
K. pneumoniae BIC	**OXA-48**	0.5	2	0.5	1×10^{1}	$>1 \times 10^{8}$	5×10^{6}
K. pneumoniae BEL	**OXA-48**	1	4	1	1×10^{1}	$>1 \times 10^{8}$	1×10^{6}
K. pneumoniae RAM	**OXA-48**	1	4	1	1×10^{1}	$>1 \times 10^{8}$	1×10^{5}
K. pneumoniae LIB	**OXA-48**	16	16	16	1×10^{1}	$>1 \times 10^{8}$	5×10^{4}
K. pneumoniae BOU	**OXA-48**	0.38	0.5	0.25	1×10^{1}	$>1 \times 10^{8}$	1×10^{8}
K. pneumoniae SCO	**OXA-48**	0.5	0.75	0.25	1×10^{1}	$>1 \times 10^{8}$	$>1 \times 10^{8}$
K. pneumoniae LOU	**OXA-48**	4	16	0.5	1×10^{1}	$>1 \times 10^{8}$	$>1 \times 10^{8}$
K. pneumoniae TIK	**OXA-48**	0.75	2	0.38	1×10^{1}	$>1 \times 10^{8}$	$>1 \times 10^{8}$
K. pneumoniae OM14	**OXA-48** + TEM-1	0.5	1	0.38	1×10^{1}	$>1 \times 10^{8}$	5×10^{7}
K. pneumoniae CHA	**OXA-48** + TEM-1	0.38	1	0.5	1×10^{1}	$>1 \times 10^{8}$	$>1 \times 10^{8}$
K. pneumoniae EGY	**OXA-48** + CTX-M-15	2	3	2	1×10^{1}	2×10^{1}	1×10^{5}
K. pneumoniae ROU	**OXA-48** + CTX-M-15	0.5	1.5	0.25	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae BEY	**OXA-48** + TEM-1 + CTX-M-15	0.38	0.38	0.38	5×10^{2}	1×10^{1}	1×10^{8}
K. pneumoniae DAL	**OXA-48** + TEM-1 + CTX-M-15	0.38	2	0.38	1×10^{1}	1×10^{1}	4×10^{5}
K. pneumoniae BAJ	**OXA-48**+ TEM-1 + CTX-M-15 + SHV-28	0.5	1.5	0.38	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae BEN	**OXA-48** + TEM-1 + CTX-M-15 + SHV-28	0.38	1	0.25	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae DUW	**OXA-48** + TEM-1 + CTX-M-15 + SHV-28	32	32	32	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae SIC	**OXA-48** + CTX-M-15 + SHV-28	0.25	1	0.25	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae AEL	**OXA-48** + CTX-M-15 + SHV-28 + OXA-1	0.5	6	0.38	1×10^{1}	1×10^{1}	5×10^{2}
K. pneumoniae AMS	**OXA-48** + TEM-1 + CTX-M-15 + OXA-1	0.5	2	0.38	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae ELK	**OXA-48** + TEM-1 + CTX-M-15 + SHV-11	0.5	3	0.38	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae VER	**OXA-48** + TEM-1 + CTX-M-15 + SHV-11	0.38	2	0.38	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae VSG	**OXA-48** + TEM-1 + CTX-M-15 + OXA-1	0.75	3	0.75	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae HPA	**OXA-48** + TEM-1 + CTX-M-15 + OXA-1	1.5	>32	12	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae OM11	**OXA-48** + TEM-1 + CTX-M-14	0.5	0.75	0.25	1×10^{1}	1×10^{1}	5×10^{7}
K. pneumoniae DIA	**OXA-48** + TEM-1b + CTX-M-15 + SHV-11 + OXA-1	>32	>32	>32	1×10^{1}	1×10^{1}	1×10^{1}
E. coli ROB	**OXA-48**	0.5	0.75	0.25	2×10^{1}	$>1 \times 10^{8}$	$>1 \times 10^{8}$
E. coli HAN	**OXA-48** + CTX-M-15	3	16	1	5×10^{1}	1×10^{1}	3×10^{4}
E. coli BOU	**OXA-48** + CTX-M-15	0.5	0.75	0.125	2×10^{1}	1×10^{1}	$>1 \times 10^{8}$
E. coli OM3	**OXA-48** + TEM-1 + CTX-M-15	0.5	1	0.38	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
E. coli OM22	**OXA-48** + TEM-1 + CTX-M-15	0.5	1	0.25	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
E. coli BER	**OXA-48** + TEM-1 + CTX-M-15	0.38	1.5	0.19	5×10^{1}	1×10^{1}	$>1 \times 10^{8}$
E. coli AME	**OXA-48** + CTX-M-24	0.25	0.5	0.19	2×10^{1}	1×10^{1}	$>1 \times 10^{8}$
E. coli ZAN	**OXA-48** + TEM-1 + CTX-M-14	0.38	8	0.75	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
E. coli BON	**OXA-48** + TEM-1 + CTX-M-24	0.38	0.5	0.19	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
E. coli BOK	**OXA-48** + CTX-M-15	0.25	0.38	0.19	2×10^{1}	1×10^{1}	$>1 \times 10^{8}$
E. cloacae TUR	**OXA-48** + SHV-5	0.5	0.5	0.5	1×10^{1}	2×10^{1}	1×10^{7}
E. cloacae 501	**OXA-48** + TEM-1 + CTX-M-15	1	16	1.5	1×10^{1}	1×10^{1}	1×10^{1}
E. cloacae BEU	**OXA-48** + TEM-1 + CTX-M-15 + SHV-12	0.5	8	0.5	1×10^{1}	1×10^{1}	1×10^{4}
C. koseri ROU	**OXA-48**	0.38	2	0.38	1×10^{1}	$>1 \times 10^{8}$	$>1 \times 10^{8}$
C. koseri VER	**OXA-48**	0.75	2	0.38	1×10^{1}	$>1 \times 10^{8}$	$>1 \times 10^{8}$
K. pneumoniae HOL	**OXA-181** + CTX-M-15	1	4	1	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae OMA	**OXA-181** + CTXM-15 + OXA-1	0.5	2	0.5	1×10^{1}	1×10^{1}	$>1 \times 10^{8}$
P. rettgeri RAP	**OXA-181** + OXA-1	8	1	2	5×10^{2}	1×10^{1}	1×10^{1}
Non-carbapenemase-producing strains							
K. pneumoniae 7725	SHV-1	0.19	0.006	0.032	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$
K. pneumoniae 0227	SHV-1	0.19	0.008	0.016	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$
K. pneumoniae 648236[e]	SHV-2a	0.25	2	0.38	1×10^{2}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae 1022	SHV-2a + SHV-28	0.5	0.016	0.023	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae BER[e]	SHV-28 + TEM-1	1	4	1	1×10^{2}	1×10^{3}	1×10^{3}
K. pneumoniae KPN	CTX-M-15	0.12	0.012	0.012	1×10^{7}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae 10112	CTX-M-15 + TEM-1 + SHV-11	0.5	0.016	0.023	6×10^{7}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae 1025	CTX-M-14 + TEM-1 + SHV-11	0.12	0.016	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae MEK[e]	CTX-M-15 + SHV-11	1.5	>32	6	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae SIM[e]	CTX-M-15 + TEM-1 + SHV-1	8	>32	6	1×10^{1}	1×10^{1}	1×10^{2}

(Continued on following page)

TABLE 1 (Continued)

Strain	β-Lactamase content[b]	MIC (μg/ml) of antibiotic[c]			Lowest detection limit (CFU/ml) for the following medium[d]:		
		IPM	ETP	MEM	Supercarba	ChromID ESBL	CHROMagar KPC
K. pneumoniae SHM[e]	CTX-M-15 +TEM-1 + SHV-11	3	>32	3	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae COO[e]	CTX-M-15 + SHV-28	8	>32	4	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae FOS[e]	CTX-M-15 + TEM-1 + SHV-11	6	>32	>32	1×10^{2}	1×10^{1}	1×10^{1}
K. pneumoniae BED[e]	CTX-M-15 + TEM-1 + SHV-11	1.5	>32	4	1×10^{1}	1×10^{1}	1×10^{1}
K. pneumoniae SHI[e]	CTX-M-15 + TEM-1 + SHV-11	0.25	1	1	7×10^{4}	1×10^{1}	$>1 \times 10^{8}$
K. pneumoniae LEG[e]	CTX-M-15 + TEM-1 + SHV-12	0.75	>32	3	2×10^{4}	2×10^{1}	2×10^{1}
K. pneumoniae ALE[e]	CTX-M-15 + SHV-1	1	>32	4	1×10^{5}	1×10^{1}	1×10^{1}
K. pneumoniae KDH[f]	DHA-2	0.12	0.5	0.12	1×10^{2}	1×10^{1}	$>1 \times 10^{8}$
E. coli 6252	None (wild type)	0.12	0.004	0.008	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$
E. coli 6367	None (wild type)	0.19	0.006	0.012	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$
E. coli 1082	TEM-1	0.19	0.019	0.016	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$
E. coli 1034	TEM-1 + SHV-38	0.19	0.006	0.016	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$
E. coli 1048	TEM-1 + SHV-2a	0.19	0.012	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli 1008	CTX-M-1 + TEM-1	0.19	0.016	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli 10122	CTX-M-1 + TEM-1	0.19	0.016	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli 1020	CTX-M-1 + TEM-1	0.19	0.023	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli 10121	CTX-M-2	0.19	0.016	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli 1023	CTX-M-2 + TEM-1	0.12	0.016	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli E14	CTX-M-14	0.12	0.012	0.012	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli FOR	CTX-M-15	0.12	0.012	0.012	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli 1033	CTX-M-15	0.19	0.012	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli EVB	VEB-1	0.12	0.012	0.012	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli 1092	OXA-1	0.12	0.19	0.023	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli ECA	ACC-1	0.12	0.012	0.012	$>1 \times 10^{8}$	5×10^{3}	$>1 \times 10^{8}$
E. coli SYD	CMY-2	0.12	0.012	0.012	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli MET	Chromosome-encoded extended-spectrum cephalosporinase	0.12	0.012	0.012	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. coli MAR[f]	Overexpressed AmpC	16	>32	2	1×10^{2}	1×10^{1}	1×10^{1}
E. coli HB4[e] (OmpC^{-}, OmpF^{-})	None	0.12	1	0.25	1×10^{1}	$>1 \times 10^{8}$	$>1 \times 10^{8}$
E. aerogenes 1009	TEM-24	0.19	0.12	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. aerogenes 1085	TEM-24	0.12	0.19	0.023	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. cloacae 7746	None (wild type)	0.38	0.064	0.032	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$
E. cloacae 7725	None (wild type)	0.19	0.008	0.012	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$
E. cloacae 5434	None (wild type)	0.38	0.016	0.032	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$
E. cloacae 1012	TEM-1 + SHV-12	0.19	0.016	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. cloacae 1072[f]	TEM-1 + OXA-1	0.38	0.5	0.064	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. cloacae CLO	CTX-M-15	0.12	0.12	0.12	1×10^{7}	1×10^{1}	$>1 \times 10^{8}$
E. cloacae 10111[f]	TEM-1 + CTX-M-15	0.5	0.75	0.094	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. cloacae 1027	TEM-1 + CTX-M-15	0.19	0.016	0.016	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. cloacae CVB	VEB-1	0.12	0.12	0.12	1×10^{4}	1×10^{1}	$>1 \times 10^{8}$
E. cloacae 1019[f]	TEM-1	0.25	1	0.094	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
E. cloacae ARF[f]	Overexpressed AmpC	0.12	1	0.12	1×10^{7}	1×10^{1}	$>1 \times 10^{8}$
E. cloacae BLA[f]	Overexpressed AmpC	0.12	1	0.12	1×10^{7}	1×10^{1}	$>1 \times 10^{8}$
E. cloacae CON[f]	Overexpressed AmpC	0.25	4	0.25	1×10^{7}	1×10^{1}	$>1 \times 10^{8}$
E. cloacae AZA[f]	Overexpressed AmpC	0.12	1	0.12	1×10^{7}	1×10^{6}	$>1 \times 10^{8}$
C. freundii 7767	None (wild type)	0.25	0.008	0.016	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$
C. freundii 10107	TEM-1 + SHV-12	0.38	0.016	0.023	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
C. freundii 1003	CTX-M-15 + TEM-1	0.38	0.016	0.023	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
C. freundii 10135	CTX-M-15	0.38	0.016	0.023	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
C. freundii MAU[f]	Overexpressed AmpC + TEM-3	1	8	1	1×10^{5}	1×10^{1}	1×10^{5}
S. Typhimurium 1081	CTX-M-1	0.25	0.19	0.032	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
P. mirabilis 1031	CTX-M-14 + TEM-1 + SHV-11	1.5	0.047	0.032	$>1 \times 10^{8}$	1×10^{1}	$>1 \times 10^{8}$
P. mirabilis PMA	ACC-1	0.25	0.094	0.064	$>1 \times 10^{8}$	$>1 \times 10^{8}$	$>1 \times 10^{8}$

[a] The MICs of imipenem, ertapenem, and meropenem and the detection limits of Supercarba medium for 176 carbapenemase- and/or ESBL/AmpC-producing enterobacterial isolates compared to the detection limits obtained with ChromID ESBL and CHROMagar KPC media are shown. The 176 enterobacterial isolates belong to the following species: *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae*, *Serratia marcescens*, *Providencia stuartii*, *Citrobacter freundii*, *Citrobacter koseri*, *Providencia rettgeri*, *Enterobacter aerogenes*, *Salmonella enterica* serotype Typhimurium, and *Proteus mirabilis*.

[b] β-Lactamase names shown in boldface type are carbapenemases.

[c] Abbreviations: IMP, imipenem; ETP, ertapenem; MP, meropenem.

[d] Underlined CFU counts are considered negative results (cutoff values set at $\geq 1 \times 10^{3}$ CFU/ml).

[e] Reduced susceptibility to ertapenem due to porin deficiency.

[f] Reduced susceptibility to ertapenem due to overexpressed AmpC.

were counted after 24 h of culture at 37°C. The sensitivity and specificity cutoff values were set at 1×10^{3} CFU/ml, i.e., a limit value of 1×10^{3} CFU/ml and above was considered "not efficiently detected."

The lowest limit of detection of OXA-48, OXA-181, NDM-1, and KPC producers ranged from 1×10^{1} to 1×10^{2} CFU/ml (Table 1). A single NDM producer (NDM-1-producing *Providencia stuartii* isolate [19]) was not efficiently detected on the Supercarba medium (detection limit of 1×10^{7} CFU/ml) (Table 1). Its lack of detection might be explained by its low MIC value of er-

TABLE 2 Sensitivity and specificity of Supercarba, ChromID ESBL, and CHROMagar KPC media

Sensitivity or specificity	Value for sensitivity (%) or specificity (%) on the following medium:		
	Supercarba	ChromID ESBL	CHROMagar KPC
Sensitivity	95.6	87.7	40.3
Specificity	82.2	24.2	85.5
Sensitivity for Ambler class of carbapenemase[a]			
Class A	100	100	66.7
Class B	90	98	55.8
Class D	100	70	13.6

[a] Sensitivity was determined for each Ambler class of carbapenemase: class A carbapenemases are of the KPC type, class B carbapenemases are of the VIM, IMP, and NDM types, whereas class D carbapenemases are of the OXA-48 type.

tapenem (0.38 μg/ml) and a likely weak expression of the $bla_{\text{NDM-1}}$ gene, related to chromosomal insertion of the $bla_{\text{NDM-1}}$ gene. As expected, OXA-181-producing *K. pneumoniae* was also detected well with the Supercarba medium. The lowest limit of detection of VIM and IMP producers ranged from 1×10^1 to 1×10^6 CFU/ml (Table 1). Although the addition of zinc sulfate significantly decreased the detection limits for VIM and IMP producers, a few VIM and IMP producers were not efficiently detected on this medium (detection limit of $\geq 1 \times 10^3$ CFU/ml). As expected, growth of isolates that do not express any carbapenemase (i.e., AmpC and/or ESBL producers) were inhibited by the Supercarba medium (with a detection limit much higher than 1×10^3 CFU/ml). In particular, the addition of cloxacillin prevented growth of the isolates expressing cephalosporinases (Table 1). As previously shown, a porin defect resulting in a decreased outer membrane permeability leads to a reduced susceptibility to ertapenem of *E. coli* and *K. pneumoniae* (7, 9, 10). In this study, among the 19 non-ertapenem-susceptible isolates with MIC values of ertapenem of >0.25 μg/ml (1 *Citrobacter freundii* isolate, 2 *E. coli* isolates, 4 *Enterobacter cloacae* isolates, and 12 *K. pneumoniae* isolates) and for which a porin defect was involved in ertapenem resistance, 58% ($n = 11$) were detected by selection on the Supercarba medium (lower detection limit of $\leq 10^2$ CFU/ml) (Table 1). The addition of zinc sulfate and cloxacillin was useful for prevention of growth of many non-carbapenemase-producing carbapenem-resistant isolates (up to 42%; $n = 8$). Noticeably, non-carbapenemase-producing *Acinetobacter baumannii* and *Pseudomonas aeruginosa* grew on the Supercarba medium (data not shown). Similar growth results of nonenterobacterial Gram-negative rods were obtained using the ChromID ESBL and CHROMagar KPC media (data not shown). These three media are suitable only for selection of members of the *Enterobacteriaceae*.

A comparison of the results obtained with the ChromID ESBL and CHROMagar KPC media with those obtained with the Supercarba medium showed that the latter screening medium is more efficient in detecting carbapenemase-producing isolates (Tables 1 and 2). Indeed, the sensitivity of the Supercarba medium was 95.6%, which was higher than the sensitivity of the ChromID ESBL (87.7%) medium and of the CHROMagar KPC (40.3%) medium. Moreover, the sensitivities of the Supercarba medium determined for each class of carbapenemase producers was higher (100%, 90%, and 100% for classes A, B, and D, respectively) than those obtained for the two other screening media (Table 2). The specificity of the Supercarba medium was also high (82.2%). A further improvement of the Supercarba medium would be the addition of chromogenic molecules that would permit recognition of species.

To assess the storage ability of the Supercarba medium, *E. cloacae* ARF that overexpressed AmpC was subcultured daily onto Drigalski agar plates from a single batch of Supercarba medium stored at 4°C. Growth of this isolate was consistently inhibited on the Supercarba agar during a 7-day period.

We propose here the very first screening medium that may detect not only KPC and MBL producers but also OXA-48 producers. This medium represents a significant improvement compared to the available screening media to detect carbapenemase producers, and particularly for detection of OXA-48 producers that do not coexpress any ESBL. Taking into account the fact that Supercarba medium contains ertapenem at a low concentration, using this medium may detect carbapenemase producers with low-level resistance to carbapenems, which is a situation frequently observed for OXA-48 producers. In addition, this medium is useful for selecting specifically carbapenemase producers in stools that also contain a large amount of ESBL producers and inhibiting the growth of ESBL producers. This property is particularly relevant, since high rates of ESBL carriage are now reported worldwide (18).

Finally, a further improvement of the Supercarba medium would be the addition of chromogenic molecules for identification of enterobacterial species.

ACKNOWLEDGMENT

This work was funded by a grant from the INSERM (UMR914), Paris, France.

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